

DEPARTMENT OF FOOD & INDUSTRIAL MICROBIOLOGY

STUDY MATERIAL

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LECTURE-1

History of Food spoilage, Food poisoning, Food legislation and Food preservation. Dietary toxins-Food poisoning, Intoxication, Infection, Classification of toxins.

Food Spoilage

1659 - Kircher demonstrated the occurrence of bacteria in milk; Bondeau did the same in 1847.

1680 – Leeuwenhoek was the first to observe yeast cells.

1780 – Scheele identified lactic acid as the principal acid in sour milk.

1836 – Latour discovered the existence of yeasts.

1839 - Kircher examined slimy beet juice and found organisms that formed slime when grown in sucrose solutions.

1857- Pasteur showed that the souring of milk was caused by the growth of organisms in it.

1873 – The first reported study on the microbial deterioration of eggs was carried out by Gayon
-Lister was first to isolate *Lactococcus lactis* in pure culture.

1876 – Tyndall observed that bacteria in decomposing substances were always traceable to air, substances, or containers.

1888 – Miquel was the first to study thermophilic bacteria.

1895 – The first records on the determination of numbers of bacteria in milk were those of Von
geuns

1902–The term psychrophile was first used by Schmidt-Nielsen for microorganisms that grow at 0°C.

1912 – The term osmophile was coined by Richter to describe yeasts that grow well in an environment of high osmotic pressure.

1915 – *Bacillus coagulans* was first isolated from coagulated milk by B.W.Hammer.

Food poisoning

1820 – The German poet Justinus kerner described “sausage poisoning” (which in all probability of was botulism) and its high fatality rate.

1888 – Gaertner first isolated *Salmonella enteritidis* from meat that had caused 57 cases of food poisoning.

1894 – T. Denys was the first to associate *Staphylococci* with food poisoning.

1896 – Van Ermengem first discovered *Clostridium botulinum*.

1906 – *Bacillus cereus* food poisoning was recognized. The first case of diphyllbothriasis was recognized.

1926 – The first report of food poisoning by streptococci was made by Linden, Turner, and Thom.

1945 – MC Clung was the first to prove the etiologic status of *Clostridium perfringens* (welchii) in food poisoning.

1951 – *Vibrio parahaemolyticus* was shown to be an agent of food poisoning by T. Fujino of Japan.

1960 – The production of aflatoxins by *Aspergillus flavus* was first reported.

1971 – First U.S. food borne outbreak of *Vibrio parahaemolyticus* gastroenteritis occurred in Maryland.

- First documented outbreak of *E. coli* food borne gastroenteritis occurred in the United States.

1985 – The irradiation of pork to 0.3 to 1.0 KGY to control *Trichinella spiralis* was approved in the United States.

1986 – Bovine spongiform encephalopathy (BSE) was first diagnosed in cattle in the United Kingdom.

Food Legislation

1890 – The first national meat inspection law was enacted. It required the inspection of meats for export only.

1906 – The U.S. Federal food and drug act was passed by congress.

1954 – The Miller pesticide chemicals amendment to the food, drug, and cosmetic act was passed by congress.

1957 – The U.S. Compulsory poultry products law was enacted.

1963 – The U.S. Food and drug administration approved the use of irradiation for the preservation of bacon.

1969 – The U.S. food and drug administration established an allowable level of 20 ppb of aflatoxin for edible grains and nuts.

Food preservation

1782 – Canning of vinegar was introduced by a Swedish chemist.

1810 – Preservation of food by canning was patented by Appert in France.

- Peter Durand was issued a British patent to preserve food in “glass, pottery, tin, or other metals, or fit materials.” The patent was later acquired by Hall, Gamble, and Donkin, possibly from Appert.

1825 – T. Kensett and E. Dagget were granted a U.S. patent for preserving food in tin cans.

1835 – A patent was granted to Newton in England for making condensed milk.

1837 – Winslow was the first to can corn from the cob.

1839 – Tin cans came in to wide use in the united states.

1840 – Fish and fruit were first canned.

1845 – S.Elliott introduced canning to Australia.

1853 – R. Chevallier-Appert obtained a patent for sterilization of food by autoclaving.

1854 – Pasteur began wine investigations. Heating to remove undesirable organisms was introduced commercially in 1867-1868.

1890 – The commercial pasteurization of milk was begun in the United States.

- Mechanical refrigeration for fruit storage was begun in Chicago.

1895 – The first bacteriological study of canning was made by Russell.

1907 – E.Metchnikoff and co-workers isolated and named one of the yogurt bacteria, *Lactobacillus delbrueckii_subsp.bulgarius*.

1908 – Sodium benzoate was given official sanction by the United States as preservative in certain foods.

1916 – The quick freezing of food was achieved in Germany by R. Plank, E. Ehrenbaum, and K.Reuter.

1928 – The first commercial use of controlled-atmosphere storage of apples was made in Europe (first used in New York in 1940).

1929 – A patent issued in France proposed the use of high-energy radiation for the processing of foods.

1943 – B.E. proctor in the United States was the first to employ the use of ionizing radiation to preserve hamburger meat.

LECTURE-2

**Food borne bacterial toxins- *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus*-
Types of food involved - toxicity and symptoms-Chemical properties- Environmental conditions.**

Food Poisoning:

Illness caused both by the ingestion of toxins as well as organism resulting from the infection through the intestinal tract.

Intoxication:

Illness caused by the consumption of a bacterial toxin formed in the food. Ex: *Clostridium botulinum*, *Staphylococcus aureus*

Infection:

Illness caused by the entrance of the bacteria into the body through ingestion of contaminated food and reaction of the body to their presence or to their metabolites.

Classification of Toxins:

1. Based on their production

a. Endotoxin: These are soluble proteins found in cell extracts or in the growth medium called as endotoxins or endogenous toxins.

Ex Lipopolysaccharide components of the outer membranes of Gram –ve bacteria.

b. Exotoxins: These are soluble proteins secreted outside the bacteria through their cell wall and these substances are heat labile, being inactivated by boiling.

2. Based on site of action

A. Enterotoxin: These toxins stimulate cells of gastrointestinal tract in an abnormal way.

Ex: Cholera toxin – *Vibrio cholerae*
Heat labile enterotoxin(LT) -- *Escherichia coli*
Heat stable enterotoxin (St) -- *Escherichia coli*
Shiga toxin -- *Shigella dysenteriae*

B. Neurotoxins: These toxins interfere with normal transmission of nerve impulses.

Ex: Botulinum toxin *Clostridium botulinum*
Tetanus toxin *Clostridium tetani*

C. Cytotoxins: These toxins affect the host cells by enzymatic attack

Ex: Diphtheria toxin -- *Corynebacterium diphtheriae*
Streptolysin O and Streptolysin S -- *Streptococcus pyogenes*

BACTERIAL TOXINS

BOTULISM

Botulism is a disease caused by the ingestion of food containing the neurotoxin produced by the *Clostridium botulinum*.

Clostridium botulinum is an anaerobic Gram positive spore forming and rod shaped bacterium. It is widely distributed in soils, fresh water and marine sediments. It produces neurotoxin (BoNT- Botulism Neuro Toxin). Other sps of *Clostridium* producing neurotoxins are *Clostridium butyricum* and *Clostridium baratii*.

Incidence of Botulism: Incidence of Botulism occurred between 1970-1973, 1899-1949.

Based on their serological properties, BoNTs are classified into 6 types.

A, B, E, & F types cause disease to humans.

Human pathogenic strains are divided into three groups.

Group I – Proteolytic and Mesophilic. Include Type A, B, F

Group II – Non proteolytic and Psychrotrophic. Include Type E and non proteolytic of strains of B & F.

Group III: Includes types C & D; they are non proteolytic and share a common metabolic pattern.

Common forms of Human Botulism:

- 1. Classical food borne botulism:** It is being an intoxication following ingestion of BoNT performed in food.
- 2. Infant Botulism:** It is an infection resulting from *Clostridium botulinum* spores germinating, growing and producing toxins in child's intestine. It results in floppy baby syndromes.
- 3. Wound botulism:** Spore germination and subsequent growth and toxin formation in deep wounds.
- 4. Adult infectious botulism:** A condition equal to infant botulism that may follow heavy antibiotic treatments or abdominal surgery.
- 5. Inhalation botulism:** Use of botulinum toxin as a therapeutic agent.
- 6. Iatrogenic botulism:** Use of botulinum toxin as a therapeutic agent.

Effect of Toxin On humans:

Initial symptoms of ingestion of toxic food occur between 12-10 days of longer.

Early symptoms are generally nausea, vomiting, followed by neurological symptoms such as double vision and fixed dilated pupils, speech impediment and difficulty in swallowing.

Ingestion of botulinal toxins results in muscle paralysis by blockade of the release of neuromuscular synapse.

Prevention of Out Break:

1. Use of approved heat processes for canned foods.
2. Rejection of all gassy (swollen) or otherwise spoiled canned foods.
3. Refused even to taste a doubtful food.
4. Avoidance of foods that have been cooked held and not well reheated.
5. Boiling of a suspected food for at least 15 min.

STAPHYLOCOCCAL GASTROENTERITIS:

Staphylococcal gastroenteritis is caused by the ingestion of food that contains one or more enterotoxins.

The bacterium responsible for this gastroenteritis is *Staphylococcus aureus*.

Staphylococcus aureus is a Gram-positive coccus forming spherical to ovoid cells about 1 µm in diameter. These are facultative anaerobes. It is a typical mesophile with a growth temperature between 7 and 48°C and an optimum temperature is 37°C. Growth occurs at pH 6-7. *Staphylococcus* occurring in masses like clusters or grapes or in pairs and short chains. Growth on solid media usually is golden or yellow but may be unpigmented in some strains. *Staphylococcus aureus* produces 6 serologically distinct enterotoxins (A, B, C1, C2, d and E)

A is most poisoning.

Enterotoxin producing species are *S. aureus*, *S. intermedius*, *S. hyicus*, *S. delphini*, *S. caprae*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. warneri*

The staphylococcal sps are host adapted inhabiting humans solely (*S. cohnii sub sp cohnii*) or humans or other animals (*S. aureus*).

Enterotoxins: Staphylococcal enterotoxins are simple proteins with molecular weights 26000-30,000. Single polypeptide chains are crosslinked by a disulfide bridge to form a characteristic cystine loop. Of the several types of enterotoxins A & D are more often associated with food poisoning outbreaks. More toxin production present at 15.6 - 46.1°C. Enterotoxins are heat stable.

Disease: Symptoms are salivation, nausea, vomiting, retching, abdominal crampings, diarrhea. Blood & mucus may be found in stools and vomiting in severe cases.

Head ache, muscular cramping, sweating, chills, prostration, Weak pulse, shock and shallow respiration may occur.

Conditions necessary for outbreak:

The following conditions are necessary for an outbreak of Staphylococcus food poisoning:

1. The food must contain enterotoxin – producing staphylococci
2. The food must be a good culture medium for growth and toxin production by the staphylococci
3. The temperature must be favourable to growth of the cocci, and enough time must be allowed for production of enterotoxin, and
4. The enterotoxin-bearing food must be ingested.

Prevention of Outbreaks:

The means of prevention of outbreaks of staphylococcus food poisoning include

1. Prevention of contamination of the food with the staphylococci,
2. Prevention of the growth of the staphylococci, and
3. Killing staphylococci in foods. Contamination of foods can be reduced by general methods of sanitation, by using ingredients free from the cocci, e.g., pasteurized rather than raw milk, and by keeping employees away from foods when these workers have staphylococcal infections in the form of colds, boils, carbuncles, etc. growth of the cocci can be prevented by adequate refrigeration of foods and, in some instances, by adjustment to a more acid pH. Also the addition of a bacteriostatic substance, such as serine or an antibiotic, has been suggested. Some foods may be pasteurized to kill the staphylococci before exposure of the foods to ordinary temperatures, e.g., pasteurization of custard filled puffs and éclairs for 30 min at 190.6 to 218.3° C oven temperature.

Clostridium perfringens:

First reported in U.S. in 1945. It causes serious wound infection. It causes gas gangrene. It is a gram positive, rod shaped anaerobe, which forms oval subterminal spores. Optimum temperature is 43 to 47°C. Optimum pH is 6.0-7.5. It produces enterotoxin. Foods involved are meats that have been cooked and allowed for cooling before consumption.

It causes gas gangrene. Enterotoxin is released in the gut during sporulation of cells and results in excessive fluid accumulation in the intestinal lumen. Food poisoning is generally characterized by nausea, abdominal pain in the intestinal lumen. Food poisoning is generally characterized by nausea, abdominal pain, diarrhoea and vomiting.

Prevention of outbreak: 1. Adequate and rapid cooling of cooked foods.

2. Holding hot foods above 60°C. 3. Reheating of leftover foods. 4. Good personal hygiene.

LECTURE-3

Food borne bacterial toxins-*Salmonella*, *Vibrio*, *Escherichia coli*, five groups of *E.coli*, *Bacillus cereus*. Types of food involved - toxicity and symptoms-Chemical properties- Environmental conditions.

Vibrio

Vibriosis: Vibriosis caused by *V. parahaemolyticus*. It causes gastroenteritis. Mainly contamination from sea food products. Natural habitat of *Vibrio* sps is sea.

Vibrio parahaemolyticus is known to cause extra intestinal infections in humans.

Vibrio consists of 28 species. 3 species are always associated in aquatic environments and seafood are *V.vulnificus*, *V.alginolyticus* and *V. cholera*

Vibrio parahaemolyticus is common in oceanic and coastal waters. Its detection is related to water temperatures; detected at 19-20°C of water. It cannot tolerate the hydrostatic pressures of ocean depths.

Growth conditions:

V.parahaemolyticus can grow in the presence of 1-8%NaCl, best growth at 2-4% range. It dies off in distilled water. It does not grow at 4°C, but growth between 5-9°C at pH 7.2-7.3 is observed in food products at higher concentrations of Nacl (3-7%). optimum temperature is 30-35°C. Optimum pH 7.6-8.6.

Virulence properties:

Most widely used in vitro test of potential virulence for *V.parahaemolyticus* is the *Kanagawa reaction*. Virulent strains are designated as k^+ and non virulent strains are k^- . K^+ strains produce thermo stable direct hemolysin (TDH); k^- strains produce a heat stable hemolysin.

Thermo stable related hemolysis (TRH) is shown to be an important virulence factor for at least some *v. parahaemolyticus* strains.

Kanagawa reaction is determined generally by use of human red blood cells in *wagatsuma's agar medium*.

To determine the k^- reaction, the culture is surface plated, incubated at 37°C for 18-24 hours and read for the presence of beta hemolysis.

Gastroenteritis syndrome

The identity of *Vibrio parahaemolyticus* as a food borne gastroenteritis agent was made first by Fujino (1951). 1951 first outbreak occurred in Japan.

Symptoms: Symptoms appear after ingestion of 3-76 hours. Diarrhea, Cramps, weakness, nausea, chills, headache and vomiting.

Vehicle foods or carrier foods are oysters, shrimps, crabs, lobsters, clams and shellfish.

Vibrio cholera: *Vibrio cholera* is best known as the cause of human cholera. Strains that cause epidemic/pandemic cholera belonged to serovar O group 1.

Vibrio vulnificus: It is found in sea water and some foods. It is isolated more often from oysters and clams.

Vibrio alginolyticus: It is also found in sea water and causes soft tissue and ear infections in humans.

Vibrio hollisae also produces enterotoxin and causes cholera.

Escherichia coli

Escherichia coli was established as a food borne pathogen in 1971 when imported cheeses were contaminated with an entero invasive strain.

E.coli is used as **an indicator for fecal contamination of water**.

Based on disease syndromes and characteristics and also on their effect on certain cell cultures and serological groupings, five virulence groups of *E. coli* are recognized:

Entero aggregative *E.coli* or Facultative entero pathogenic *E.coli*

Entero hemorrhagic *E.coli* (EHEC)

Entero invasive *E.coli* (EIEL)

Entero pathogenic *E. coli* (EPEC)

Entero toxigenic *E. coli* (ETEC)

This organism was first isolated and described by German bacteriologist Theodor Escherich from children's faeces in 1885.

E. coli is an almost universal inhabitant of the gut of humans and other warm blooded animals where it is the predominant facultative anaerobe through only a minor component of the total micro flora.

E. coli sero type 0157:47 has been recognized as the cause of a number of out breaks of haemorrhagic colitis and haemolytic uraemic syndrome.

E.coli is a catalase positive, oxidase negative, fermentative, short Gram negative, non spore forming rod. Genetically *E. coli* is closely related to the genus *Shigella*, although characteristically it ferments the sugar lactose and is otherwise for more active biochemically than *Shigella* sps.

IMVIC test is used to differentiate from other members of the Enterobacteriaceae.

I = Indole from tryptophan

M = sufficient acid to reduce the medium pH below 4.4, the break point of the indicator methyl red (M).

V = Acetoin (acetyl methyl carbinol); C = the ability to utilize citrate

E. coli is a typical mesophile growing from 7-10°C up to 50°C (opt temp 37°C)

It produces both gas and acid on carbohydrate. It causes off flavor, gassiness, off odor described as "barny".

1. Enteropathogenic *Escherichia coli* (EPEC): They do not produce enterotoxins but causes diarrhea.
 2. Enteroinvasive *Escherichia coli* (EIEC): It does not produce enterotoxins. It causes ulceration and inflammation in the epithelial cells of the colon. It causes symptoms of invasive bacillary dysentery normally associated with *Shigella*.
 - 3) Enterohaemorrhagic *E. coli* (EHEC): Produces two basic toxins. Usually they are called shiga like toxin SLT-I SLT-II. Also known as Vero toxin producing *E. coli* (VTEC).
 - 4) Enterotoxigenic *E. coli* (ETEC): Primary enterotoxins which are heat labile and stable are produced.
 - 5) Facultative enteropathogenic *E. coli* (FEEC): It is associated with sporadic diarrhea outbreaks.
- Eosin methylene blue agar is a popular selective and differential medium for isolation and identification of *E. coli* from suspected samples

Enterotoxins:

Enterotoxins are heat labile (LT) and other is heat stable (STa or ST-I and STb or ST-II). The LT toxin is destroyed at 60°C in about 30 minutes whereas ST toxins can withstand 100°C for 15 minutes.

LT toxins are a protein with a molecular weight of about 91 kDa and it possesses enzymatic activity similar to that of the cholera toxin (CT).

CT is exported from the cytoplasm to the outside of producing cells; LT is deposited in to the Periplasm of producing cells.

Prevention:

1. Proper cooking of food before consumption. Eg: Beef should be cooked to 160°F or 71.7°C

Traveler's Diarrhea: *E. coli* is well established as one of the leading causes of acute watery diarrhea that often occurs among new arrivals in certain foreign countries caused by

ETEC strains, EPEC and ST producing strains. Among other organisms associated with this syndrome are rotaviruses, noro viruses, *Entamoeba histolytica*, *Yersinia enterocolitica*, *Giardia lamblia*, *Campylobacter jejuni/coli*, *Shigella*, *sps. Aeromonas hydrophila*, *Klebsiella pneumoniae*, *Enterobacter cloacae*.

Bacillus cereus

Bacillus cereus is an aerobic, spore forming rod normally present in soil, dust and water. It has been associated with food poisoning in Europe since at least 1906.

Plazikowski first reported this syndrome.

First documented outbreak in the United States occurred in 1969 and also in Great Britain in 1971. Other *sps* of *Bacillus* producing enterotoxins are *Bacillus circulans*, *Bacillus lentus*, *B. polymyxa*, *B. carotaneum*, *B. pasteurii*, *B. mycoides*.

B. mycoides strains from milk have been shown to produce diarrheagenic enterotoxin.

B. thuringiensis has been isolated from foods and it produces Vero-cell active toxin.

This bacterium has a minimum growth temperature around 4-5°C with a maximum around 48-50°C. Growth observed at pH range 4.9-9.3.

It produces a wide variety of extra cellular toxins, enzymes, including lecithinase, preteases, β -lactamase, sphingomyelinase, cereolysin and hemolysin BL.

Cereolysin is a thiol activated toxin analogous to perfringolysin O and causes food borne gastroenteritis.

Bacillus cereus virulence factor is a hemolytic enterotoxin complex designated HBL and it exhibits hemolysis, dermonecrosis and vascular permeability properties.

Diarrheal syndrome:

This syndrome is rather mild with symptoms developing within 8-16 hours, more commonly within 12-13 hours. Symptoms consist of nausea, cramp like abdominal pains, tenesmus and watery stools.

Emetic syndrome: Also called as Chinese restaurant syndrome because its association with starchy products like rice and dishes. This form of *Bacillus cereus* food poisoning is more severe and acute than the diarrheal syndrome. It forms similar to Staphylococcal food poisoning syndrome.

It is often associated with fried or boiled rice dishes, pasteurized cream, spaghetti, mashed potatoes etc. The emetic toxin has been determined to be cereulide, an ionophoric water insoluble peptide that is closely related to the peptide antibiotic valinomycin.

LECTURE-4

Food borne bacterial toxins-*Listeria*, *Shigella*, *Yersinia*, *Campylobacter*, *Aeromonas*, *Brucella*, *Pleisiomonas*. Types of food involved - toxicity and symptoms-Chemical properties-Environmental conditions.

Listeriosis

Listeriosis caused by *Listeria monocytogenes*. Listeriae are Gram positive, non spore forming and non acid fast rods.

Primary pathogenic species *Listeria monocytogenes*, *Listeria innocua*, *Listeria seeligeri*

They grow well in many common media such as brain heart infusion, trypticase soy and tryptose broths. Opt pH 6.0-8.0.

Generally Listeriae are widely distributed in nature and in soils, animal feces, sewage, silage and water.

Fresh food product of animal or plant origin may harbor varying numbers of *L.monocytogenes*. In general the organism has been found in raw milk, soft cheeses, fresh and frozen meat, poultry and sea food products. Listeriolysin O is produced during exponential grown phase. Listeriolysin is produced by *L. monocytogenes*, *L.welshii* or *L.grayi*.

Ivanolysin O is produced by *Listeria ivanovii* and *L. seeligeri*. It is not identical to Listeriolysin.

Symptoms:

Incubation period for the disease have varied from one day to 90 days. Pregnant women are more susceptible to non pregnant humans. In pregnant women symptoms include mild flu like illness to meningitis and meningoencephalitis, fever, headache, occasional gastro intestinal symptoms but there may be an associated transplacental foetal infection which can result in abortion, still birth or premature labour.

Listeriosis in the new born babies observed and infection occurs from *in utero*, possibly through the aspiration of infected amniotic fluid and characterized by pneumonia, septicemia and widely disseminated granulomas (abscesses). Meningitis is rare. Listeriosis in non pregnant adults is usually characterized by septicaemia, Meningitis and meningoencephalitis, but can also include endocarditis.

First outbreak of Listeriosis occurred in Maritime Provinces of Canada in 1981. *Listeria monocytogenes* isolated from a sample of Cole slaw in a patient's refrigerator. Raw vegetables, dairy foods, beet, meat, cheese act as the source of contamination.

Regulatory status of *L.monocytogenes*: Zero absence in 50g sample.

Zero tolerance generally means the absence of the organism in 25g sample which is equivalent to $n=5$, $c=0$ in an amply plan.

European community (EC) directive on milk and milk based products specifies zero tolerance for soft cheeses, and absence of the organism in 1g of other guide lines for ready to eat foods establishes four quality groups based on numbers of *L.monocytogenes*.

1. Satisfactory –Not detected in 25g sample.
2. $10^2/25g$ sample – fairly satisfactory
3. $10^2-10^3/25g$ sample – unsatisfactory
4. $>10^3/25g$ sample – unacceptable.

ICMSF –International Commission on Microbiological Specification for foods (ICMSF) has concluded that if this organism does not exceed 100/g of food at point of consumption. The food is considered acceptable for individuals.

Salmonellosis

Salmonellosis caused by *Salmonella* sps. Salmonellae are small, Gram negative, non sporing rods. They are widely distributed in nature, with humans and animals being their primary reservoirs. *Salmonella* have placed in two species.

Salmonella enterica and *Salmonella bongori*.

Widal test is used to detect the *Salmonella*.

For epidemiological purposes, the *Salmonella* can be placed into three groups.

1. Those infect humans only - *S.typhi*, *S. paratyphia*, *S. paratyphic*. It includes the agents of typhoid and the paratyphoid fevers, which are the most severe of all diseases caused by salmonella.
2. The host adapted serovars.

Ex: *S.enterica Gallinarum* (poultry), *S. enterica Dublin* (cattle), *S. abortus-equi* (Horses)*S. enterica Abortus- ovis* (sheep) *S.enterica choleraesuis* (swine).

3. Unadapted serovars (no host preference):

These are pathogenic for humans and other animals. They include most food borne serovars.

Distribution:Primary habitat of *Salmonella* sps is the intestinal tract of animals such as birds, reptiles, farm animals, humans and occasionally insects. Also occurs in animal feeds, commercially prepared and packaged foods.

Growth and Destruction of Salmonella: Temp - 37°C; pH - 4.0-9.0. With respect to destruction, pasteurization temperature is enough to kill salmonellae. Ionizing radiation with doses of 5-7.5 KGy being sufficient to kill salmonellae.

Kauffman – White serotyping scheme has proved the most useful technique for differentiating within the genus. This describes organisms on the basis of their somatic (O) and flagellar (H) antigens and by Capsular (vi) antigens possessed by *Salmonella typhi*, *Salmonella Dublin*, *Salmonella paratyphi C*

Salmonellae Food poisoning Syndrome:

This syndrome is caused by the ingestion of foods that contain significant numbers of non host specific or sero types of the genus *Salmonella*. Symptoms usually develop in 12-14 hours. Symptoms consist of nausea, vomiting, abdominal pain, headache, chills, diarrhea. These symptoms are usually accompanied by prostration, muscular weakness, faintness, moderate fever, restlessness and drowsiness.

Among the different sps of *S. enterica choleraesuis* has been reported to produce the highest mortality rate. *S. enterica Enteritidis* –common in eggs

Prevention:

1. Raw or Undercooked eggs should be avoided.
2. When eggs are not properly cooked, pasteurized egg products should be used.
3. Eggs should be cooked at $\geq 145^{\circ}\text{F}$ (67°C) for 15 seconds.
4. Raw eggs should be stored at $\leq 45^{\circ}\text{F}$ (7.2°C)

Shigellosis:

Shigellosis caused by *Shigella* sps. Belongs to the family Enterobacteriaceae.

S. dysenteriae, *S. flexneri*, *S. boydii* and *S. sonnei*

The genus *Shigella* was discovered as the cause of bacillary by the Japanese microbiologist Kiyoshi Shiga in 1898. They are non motile, no spore forming, Gram negative, and facultative anaerobes.

Temperature required for growth 10-45°C. They grow best in the pH range 6-8.

Pathogenesis:

Shigella causes dysentery in humans and other higher primates. Incubation period 7 hours to 7 periods. Symptoms are of abdominal pain, vomiting and fever accompanying a diarrhea which can range from a classic dysenteric syndrome of bloody stools containing mucus and pus. Source of contamination from sewage, human faeces, sea foods. etc.

Yersiniosis

Yersinia pestis causes plague. *Yersinia enterocolitica* –commonly found in foods. First isolated in New York State in 1933 by Coleman. It is Gram negative rod and psychotropic.

Growth requirements: Growth of *Y. enterocolitica* has been observed over the temperature range - 2°C to 45°C, pH 4.0-5.0. It is distributed in the terrestrial environment lake, well, stream waters.

Y. enterocolitica, *Y.intermedia*, *Yersinia freder jksenii* *Y. Kristensenii* are associated with fish, fresh waters and wods.

Syndrome: It produces gastroenteritis. The organism has been recovered from urine, blood, cerebrospinal fluid and eyes of infected individuals. Symptoms include fever, diarrhea, severe abdominal pain, vomiting, headache. Milk is a common vehicle food.

Campylobacter

The syndrome caused by *Campylobacter* is campylobacteriosis caused by *C. JeJuni sub sp. Jejuni* – It is reffered to as *C.Jejuni*. *C.Jejuni sub sp Jejuni*. It is resistant to cephalothin

Campylobacters were known primarily to cause abortions in cattle and sheep.

C.Jejuni is a slender, spirally curved rod that possesses a single polar flagellum at one or both ends of the cell. It is microaerophilic, requiring small amounts of oxygen (3-6%) for growth.

Temperature required is 30-45°C. Grows in the pH range of 5.5-8.30.

Distribution: It is not an environmental organism but associated with warm blooded animals. A large percentage of all major meat animals have been shown to contain these organisms in their feces. *C.Jejuni* produces a heat labile enterotoxin (CJT).

Enteritis syndrome caused by *C.Jejuni* and includes symptoms like abdominal cramps, diarrhea, malaise, headache and fever.

Prevention:

1. They destroyed by pasteurization temperatures.
2. Drinking water should be properly treated.
3. Avoid eating of under cooked or unpasteurized foods of animal origin like milk and chicken.

Aeromonas:

Currently, *Aeromonas* has the status of a food borne pathogen of emerging importance.

It has the ability to grow at low temperatures.

Aeromonas hydrophila, *Aeromonas sobria*; *Aeromonas caviae*.

Aeromonas salmonicida is not a human pathogen but causes diseases of fresh water fish.

Aeromonas may also cause “travelers diarrhea”.

Aeromonads are Gram negative, catalase positive, oxidase positive rods. They are motile by a single polar flagellum. Optimum temperature is 28°C.

Aeromonas causes gastroenteritis in children under five years old. Symptoms include watery diarrhea, dysenteric stools. *Aeromonas hydrophila* and *Aeromonas sobria* produces aerolysin. Aerolysin is heat labile, β - haemolytic, cytotoxic entero toxin.

Brucella

The genus *Brucella* is named after Sir David Bruce who in 1887 recognized it as the causative organism of undulant fever (Brucellosis, Malta fever, Mediterranean fever).

Brucella abortus –cattle; *Brucella melitensis* – sheep and goats

Brucella suis – pigs; *Brucella canis* – dogs

Brucella are Gram negative, catalase positive, oxidase positive, short oval rods which are non motile and usually occur singly, in pairs or rarely in short chains. It grows optimally around 37°C and is killed by heating at 63°C for 7-10 min.

Brucellosis is a protracted and debilitating illness characterized by an incubation period from one to six weeks followed by a chronic, relapsing fever with accompanying lassitude, sweats, headache, constipation, anorexia, pains in the limbs and back and weight loss.

➤ *Brucella* are quite fastidious organism and do not grow in conventional laboratory media. Liver infusions or calf serum are normally added to the media.

Ring test:

Cattle are tested for the presence of antibodies to the organism in this test.

Stained antigen is mixed with the test milk, if antibodies to *Brucella* are present, then they will cause the antigen to clump and rise with the milk fast on standing to form an intense blue violet ring at the top of the milk. *Brucella* associated with meat, raw milk and cream.

Plesiomonas

Plesiomonas shigelloides is a short, catalase positive, oxidase positive, gram negative rod. It is motile by polar, generally lophotrichous. It grows over a temperature range from 8-10°C to 40-45°C. Fish and shellfish are a natural reservoir of the organism. Also associated with crabs, shrimps, cuttle fish and oysters. *P.shigelloides* infections are more common in warmer climates and in travelers returning from warmer climates. Usual symptoms are mild watery diarrhea free from blood or mucus.

LECTURE-5

Mycotoxins-Types of mycotoxins-Aflatoxins, Patulin, Penicillic acid, Ochratoxin, citrinin, Alternaria toxin- Types of food involved - toxicity and symptoms-Chemical properties-Environmental conditions.

Mycotoxins

Mycotoxins are produced as secondary metabolites of fungi. Some Mycotoxins are mutagenic and carcinogenic. At least 14 Mycotoxins are known to be carcinogens. Secondary metabolites are formed during end of the exponential growth phase.

Different types of Mycotoxins are

1. Aflatoxins, 2. Penicillic acid, 3. Patulin, 4. Mush room toxins, 5. Citrinin, 6. Ochratoxin
7. Alternaria toxins, 8. Sterigmatocystin, 9. Fumonisin, 10. Ambutoxin, 11. Zearalenone

Aflatoxins

Aflatoxins are clearly the most widely studied of Mycotoxins.

In 1960, 100,000 turkey poultts died in England after eating peanut meal imported from Africa and South America.

Organism responsible for aflatoxins is *Aspergillus flavus*. *A.flavus* produces AFB is the most potent of all. *A. parasiticus* produces B₁, G₁, B₂ and G₂.

Other known fungi which produce aflatoxins include *A.nominus*, *A. bombycis*, *A.pseudotamarii*, *A. ochra ceoroseus* and *Emericella venezuelensis*.

Toxin:

Toxin produced by *Aspergillus* is Aflatoxin (A- Fla –toxin). These compounds are highly substituted coumarins.

AF M₁ is a hydroxylated product of AFB₁ and it appears in milk, urine and feces as a metabolic product.

AFL, AFLH₁, AFQ₁ and AFP₁ are all derived from AFB₁.

AFB₂ is the 2, 3- di hydro form of AFB₁.

AFG₂ is the 2, 3 – di hydro form of AFG₁.

The toxicity of the six most potent aflatoxins decreases in the following order:

B₁ > M₁ > G₁ > B₂ > M₂ ≠ G₂.

G₁ When viewed under ultra violet light, toxins fluoresce as noted:

B₁ and B₂ – blue

G₁ – green

G₂ – green – blue

M₁ – Blue – violet

M₂ – Violet.

They are polyketide secondary metabolites.

Growth and Toxin production occurred at 33°C when pH was 5.0. Optimum temperature for toxin production is 24-28°C. Growth and formation of Aflatoxin occur mostly during the curing of peanuts after removal from soil.

The U.S. Food and Drug Administration (FDA) has established allowable level of aflatoxins as follows

20 ppb for food, feeds, Brazil nuts, peanuts pistachio nuts; 0.5 ppb for milk.

Codex Alimentarius Commission recommended the following maximum levels of mycotoxins in specific foods.

15 µg/Kg - pea nuts; 0.05µg/Kg M1 aflatoxin - Milk

50µg/Kg –Apple juice –patulin

5µg/Kg – cereal and cereal products –ochratoxin.

According to W .H. O and F. A. O -30 µg/Kg in foods for human consumption.

Toxicity and Mode of Action:

Aflatoxins are carcinogenic to humans.

EFDV syndrome of Thailand, Reye's syndrome of Thailand, acute hepatoma condition in Ugandan child are related to aflatoxins.

LD₅₀ values of Aflatoxin B₁ 0.35 and 0.5mg/Kg body weight of day old buckling and dog.

For rat W.H.O and F.A.O -30µg/ Kg body weight.

Degradation of Toxin:

AFB₁ and AFB₂ can be reduced in corn by bisulfite.

Heating of food product for 1 hr at 45-65° affects 68% reduction.

U.V radiations affect 45% reduction.

Patulin

Patulin (Clavacin or expansin) is produced by a large number of fungi including

Penicillium claviforme, *Penicillium expansum* *Penicillium patulum* *Aspergillus clavatus*, *Aspergillus terreus*, *Byssochlamys nivea* and *Byssochlamys fulva*.

Its biological properties are similar to those of Penicillic acid.

This mycotoxin has been found in moldy bread, sausage, fruits, apple juice, cider and other products.

LD₅₀ for Patulin in rats by the subcutaneous route is 15-20mg/Kg.

To inhibit production of patulin, SO₂ was found more effective than Potassium sorbate or sodium benzoate.

Patulin in the pure state is a white crystal with a melting point of 110.5°C and molecular weight of 154 KD. It is an unsaturated lactone with the chemical name of 4-hydroxy-4H-furo(3,2) puran-2(6H)-one.

Penicillic Acid

This mycotoxin has biological properties similar to Patulin produced by *Penicillium puberulum*, *Penicillium cyclopium*. It has been found in corn, beans and other field crops and has been produced experimentally on Swiss cheese. LD₅₀ in mice by subcutaneous route is 100-300mg/Kg.

Ochratoxin

Ochratoxin is a potent nephrotoxin was first isolated from *Aspergillus ochraceus* in South Africa. It is commonly contaminant of cereals, barley, infected with *Penicillium verrucosum*.

A.ochraceus, *A. alliaceus*, *A. ostianus*, *A. mellus*, *A. niger* and *A. carbonarius*.

Among *Penicillium viridicatum*, *Penicillium lyclopium*, *P. variable*.

Ochratoxin A is structurally a chlorinated isocoumarin derivative with an amide bond to phenyl alanine.

Ochratoxin A fluoresces green under U.V light. Ochratoxin B fluoresces blue under U. V light.

Ochratoxin A is heat stable and could not be destroyed by normal cooking processes.

Citrinin

Citrinin mycotoxin is produced by *Penicillium citrinum*, *Penicillium viridicatum*.

It has been recovered from polished rice, moldy bread, country – cured hams, wheat, oats, Nye and other similar products. Under long wave U.V light, it fluoresces lemon yellow. It is a known carcinogen.

Alternaria Toxin

Alternaria citri, *A. alternate*, *A.solani* and *Alternaria tenuissima* produces toxins found in apples, tomatoes, blue berries, grains and other foods.

The toxins produced include alternariol, alternariol monomethyl ether, altenuene, tenuazoic acid and alter toxin-I.

LECTURE-6

Mycotoxins-Sterigmatocystin, Fumonisins, Sambutoxin, Zeralenone, Ergotism, Cyclopiazonic acid, Rubratoxin, Satratoxin, Verrucarins, and Roridin, Tricothecenes, Roquefortine- Types of food involved - toxicity and symptoms-Chemical properties- Environmental conditions.

Sterigmatocystin

This mycotoxin is structurally and biologically related to the aflatoxins and like the latter, it causes hepatocarcinogenic activity in animals.

Ex: *Aspergillus versicolor*, *Aspergillus nidulans*, *Aspergillus rugulosus* and others.

LD₅₀ for rats by intraperitoneal injection is 60-65 mg per Kg body weight. Under U.V light, this toxin fluoresce dark brick red.

Fumonisins

Fumonisins are produced by *Fusarium sacchari*, *Fusarium subglutinans*, and *Fusarium thapsinum*.

Fusarium moniliforme was the first to be associated with the mycotoxin and it is the best studied of the three.

It is present in corns, grains and grain products.

Sambutoxin

It is associated with dry – rotted potatoes and is produced primarily by *Fusarium sambucinum*. This toxin causes oesophageal cancer.

Zeralenone

This toxin is produced by *Fusarium graminearum* and *F. tricinctum*. These fungi invade field corn at the silking stage, especially during heavy rain fall.

Ergot

Ergotism in humans was called St. Anthony's Fire. Ergotism is caused by the alkaloids produced by fungi *Caviceps purpurea* and *C. paspali* growing on the plants of rye and other members of the family Graminae. The symptoms of ergotism include hyperexcitability, belligerency, tremors, stumbling and collapse.

Cyclopiazonic Acid

Cyclopiazonic acid is a toxic indole tetramic acid that is produced by the numerous sps of *Aspergillus* and *Penicillium*. It is a natural contaminant of corn and peanuts and was implicated in human intoxication involving millet.

Rubratoxin

Rubratoxin produced by *Penicillium rubrum* which infects cereals and legumes. It causes haemorrhagic enterocolitis and abortion.

Island toxin, Cyclochlorotine and Luteoskyrine *Penicillium islandicum* is a storage mold found commonly in agricultural commodities. This mold produces a large number of metabolites, some of which, namely island toxin, Luteoskyrine, Cyclochlorotine and regulosin are reported to be carcinogenic.

Satratoxin, Verrucaridin and Roridin:

Fungi of the genus *Stachybotrys* are typical saprophytes found ubiquitously on hay and other cellulose rich materials. They produce a number of metabolites including Verrucaridin, Roridin, mullein and Satratoxin. The mycotoxicoses also referred to as Stachybotrytotoxicosis has been observed in horse, cattle, poultry and humans.

Trichothecenes

Trichothecenes are a group of sesquiterpenoid toxins mainly produced by the species of *Fusarium* and related genera. Diseases include Alimentary Toxic leukemia (ATL) in humans due to the consumption of cereals infected with *F.poa* and *F. sporotrichoides*.

Roquefortine

Recently detected in commercial blue cheese from several countries. Produced by *Penicillium roqueforti*. It is a neurotoxin leads to convulsive seizures.

Control of Mycotoxin in food and feed:

1. Prevention of mold growth in standing crops and post harvest storage.
2. Proper handling of a commodity without mold contamination is the best kind of control of mycotoxin.
3. Infected material should be separated and detoxification of a commodity using ammonia.
4. Cultivation of crop varieties resistance to fungal infections.
5. Cultural practices like appropriate irrigation, timely application, of fungicides and insecticides can also help control the accumulation of Mycotoxins in agricultural commodities.

LECTURE-7

Mushroom toxins, Different species of poisonous mushrooms, Poisoning or disorders due to poisonous mushrooms. Control of mycotoxins in food & feed.

Mushroom toxins:

The consumption of poisonous mushroom may cause serious illness, even death. *Amanita phalloides* and *Amanita verna* are highly toxic among the other *Amanita* spp. Poison causes paralysis of nervous system and degeneration of the liver starts.

Amanita muscaria is also a poisonous species which results in death of healthy person. After intake, persons suffer from vomiting and diarrhea, loss of memory and have tendency to sleep. Active toxic principle in it is mycetomuscarine and muscardine. Other spp of poisonous species of mushrooms which may cause severe illness or even death.

- 1) ***Gyromitra esculenta*:** The poisonous substance in this mushroom is helvellic acid which is soluble in hot water. The dried and cooled mushrooms are safe. This species can be identified with a whitish stiffed or hollow stipe, binucleate, large, yellowish spores.
- 2) ***Inocybe patouidare*:** This species can be identified by the presence of conical cap, absence of ring on the stipe and in rolled margin. This mushroom becomes brownish red during handling. The poisoning causes diarrhea, loss of memory and tendency to sleep.
- 3) ***Entoloma lividum* and *Entoloma sinuatum*:** This mushroom may be distinguished from field mushroom due to the absence of the ring on the stipe. Consumption of this mushroom causes symptoms like violet sickness and diarrhea.

Poisoning or Disorders due to poisonous Mushrooms

1. Cellular poisoning: *Amanita* species and *Galerina* species are mainly responsible for this type of poisoning. Toxin amanita attacks body tissues and cause death. Small quantity (5-10mg) or one *Amanita* cap or 15-20 small caps of *Galerina* are sufficient to kill a healthy man. This toxin is thermo stable and is not removed by boiling. This is intracellular and interferes with DNA and RNA functions. Symptoms include elevated blood chemistries and hepatic renal failure. Other symptoms include abdominal pain and ultimately kidney and liver failures. Small irregular pupils, stiffness of neck twitching of facial are often present prior to death.

2. Nerve poisoning: This type of poisoning occurs after 2 hours of eating of mushrooms. The consumption of *Amanita muscutria*, *Amanita pantherina*, *Inocybe pathotillardii*, *Caliticybe deblata*

causes this type of poisoning. The toxins muscarine and Ibotenic acid contained in these poisoning mushrooms produces excessive perspiration, salivation and lacrimation suddenly. These followed by blurring of vision and shortly thereafter accompanied by loose watery stools. Constriction of pupils, a fall in blood pressure and slow pulse cause coma and often death. The fatal death rates are 6-12%.

3. The Gastric poison: Several mushrooms named *Poletus satanus*, *Entomola lividum*, *Lactarius fornosus* and *Russula sps* cause this type of poisoning. Norcaperatic acid has been found to be the common cause of this poisoning. Symptoms are early occurrence of nausea, vomiting and diarrhea which terminate rapidly and the patient recovers in a day or two from poisoning.

Differences between poisonous and edible mushrooms

Poisonous mushrooms	Edible mushrooms
1. They have ring on its stalk. Some poisonous mushrooms have no stalk. Ex: <i>Amanita virosee</i> .	1. No ring on its stalk. <i>Agaricus campestris</i> is edible have rings on its stalk.
2. These mushrooms have swollen stalks.	2. No swollen stalks
3. White capped mushrooms should be avoided	3. Cup or suck about the base of stalk of mushrooms is not safe to consume.
4. Fleshy mushrooms with pores are not safe to consume till it is properly identified	4. Edible mushrooms have no pores.
5. They are colored (brownish)	5. They are white.

Amanita phalloides – death cap mushrooms.

4. Poisoning effect of mushroom with alcohol: This type of poisoning is reported if *Coprinus atramontamius* commonly known as ink cap is consumed with or after drinking alcohol. The symptoms occur in short time. Flushing of the face and neck with throbbing distension of neck veins, a feeling of swelling parenthesis in the hand and feet followed by chest pain. Later nausea, vomiting and sweating occurs.

5. Muscular poisoning: The muscular disorders appear after 30-60 minutes of *Psilocybe cubensis* mushroom. The symptoms appears as excitement in muscular system especially in the

smooth muscle fibres and the patient may feel muscle weakness and drowsiness may occur, This type of disorder appears due to Psilocybin toxin.

Preliminary Treatments if poisonous mushrooms are consumed:

- 1) Expulsions of the poisonous mushrooms are consumed, by vomiting as soon as possible. If vomiting is not possible give a teaspoon of mustard oil in half glass of water.
- 2) **Elimination of toxin:** The poison already absorbed in the blood may be eliminated or exhausted by subcutaneous injection of atropine and by other medical means.
- 3) **Some mushrooms affect the action of heart:** In these types of causes, doctors should be called immediately. Meanwhile stimulants can be given.
- 4) Depression, sleepiness, lethargy can be treated by the use of strong tea, coffee etc. prolonged and excessive vomiting can be checked with soda water or by giving patient a small piece of ice to suck.

Control of Mycotoxin in food and feed:

1. Prevention of mold growth in standing crops and post harvest storage.
2. Proper handling of a commodity without mold contamination is the best kind of control of mycotoxin.
3. Infected material should be separated and detoxification of a commodity using ammonia.
4. Cultivation of crop varieties resistance to fungal infections.
5. Cultural practices like appropriate irrigation, timely application, of fungicides and insecticides can also help control the accumulation of Mycotoxins in agricultural commodities.

LECTURE-8

Algal toxins-Paralytic shell fish poisoning, Ciguatera poisoning, Domoic acid, Tetrodo toxin, Cyanobacterial toxins, Scombroid Fish poisoning, *Pfiesteria piscicida*- Types of food involved - toxicity and symptoms-Chemical properties- Environmental conditions.

Algal Toxins

Paralytic Shellfish Poisoning:

This syndrome is contracted by eating toxic mussels, oysters, scallops or cockles. They become toxic after feeding on certain dinoflagellates of which *Gonyaulax catenella*.

Gonyaulax tamarensis is more toxic than *G.catenella*. Masses or blooms of these toxic dinoflagellates give rise to the red tide condition of seas.

Another dinoflagellate, *Karenia brevis* produces brevetoxin which cause respiratory distress and food poisoning in humans. It has caused massive fish kills along the east coast of the United States and has been implicated in the death of bottle nose dolphins and manatees.

Another paralytic shell fish poison (PSP) is saxitoxin. Saxitoxin exerts its effect in humans through cardiovascular collapse and respiratory failure. Detoxification of molluscs can be achieved by their transfer to clean water and a month or more may be required.

Saxitoxin- *Saxidomas giganteus*. Gonyatoxin - *G.catenella*; *G.tamarensis*.

Ciguatera poisoning

This poisoning occurs from the ingestion of fish that feed on herbivorous or reef fishes which in turn feed on dinoflagellates. The responsible dinoflagellate is *Gambier discus toxicus* which produces ciguatoxin. This toxin is concentrated more in fish organs such as the liver than in muscle tissue.

Fish feed on toxic algae *Lyngbya majascula*. Upon ingestion of toxic fish; symptoms occur within 3-6 hours and consist of nausea and parasthesia about the mouth, tongue and throat.

Domoic acid

This is an uncommon amino acid that antagonizes glutamic acid in the central nervous system. It is produced by diatom *Pseudonitzschia pungens*.

Diatoms are single celled amnesic shellfish poisoning (ASP) following the consumption of mussels or scallops harvested from marine waters with a bloom of the diatom noted.

Canadian regulatory level is 20µg/g of tissue for fresh bivalve mollusks.

***Pfiesteria piscicida*:** It is dinoflagellate that produces potent toxins. This toxin kills fish within a few seconds and the animals dies within a few minutes. It is heat stable.

Tetrodo toxin: Tetrodo toxin is present in fugu or puffer fish. This algal toxin accumulates in the gonads and viscera of the fish that feed on the Calcareous alga of *Jania* sp.

Tetrodo toxin is found to block to the sodium pump. The toxin can be avoided by eviscerating the fish before consumption.

Cyanobacterial Toxins

Several genera of fresh water cyanobacteria, especially species of *Microcystis*, *Anabaena* and *Aphanizomenon* can form extensive blooms in lakes, ponds and reservoirs and may cause deaths of animals drinking the contaminated water. The presence of such cyanobacteria in public water supplies leads to out breaks of gastroenteritis.

Cyanoginosins are toxic metabolites of *Microcystis aeruginosa* are cyclic polypeptides containing some very unusual amino acids and are essentially hepato toxins.

Scombroid toxic Fish poisoning

Scombroid toxic fish poisoning results from the bacterial action on Scombroid fish such as tuna or mackerel. Bacteria act as indirect agents of food poisoning by converting food components in to harmful compounds.

Symptoms produced due to histamine toxicity and can be alleviated with antihistamines. Histamine is produced by bacterial amino acid decarboxylases acting on histidine which occurs in high concentrations in the tissues of dark fleshed fish.

To avoid this poisoning, immediately after catching of fish stored in refrigerator.

Symptoms include dizziness, sharp and peppery taste in mouth, feverishness, diarrhea, nausea, vomiting.

LECTURE-9

Food borne animal parasites-Protozoans-Giardiasis, Amebiasis, Toxoplasmosis, Sarcocystis, Cryptosporidiosis, Cyclosporiasis- Types of food involved - toxicity and symptoms-Chemical properties- Environmental conditions.

Food borne animal parasites include protozoa, flat worms and round worms.

Protozoans

Giardiasis: *Giardia lamblia* is a flagellate protozoan that exists in environmental waters at a higher level than *Entamoeba histolytica*. These organisms produce cysts in water and foods. The trophozoites have eight flagella that arise on the ventral side near the paired nuclei and gives rise to falling leaf motility. Water is the second most common source of giardiasis.

Upon ingestion, *Giardia* cysts excyst in the gastrointestinal tracts with the aid of stomach acidity and proteases and give rise to clinical giardiasis. Symptoms include abdominal cramps, abdominal distention, nausea, weight loss. *Giardia* has been shown to occur in some vegetables and may be contaminated from water by unsanitary conditions.

Treatment by use of drugs like quinacrine, metronidazole and tinidazole.

Amebiasis: Amebiasis (amoebic dysentery) caused by *Entamoeba histolytica*. It is often transmitted by the fecal oral route, water, food handlers and foods.

Symptoms include loose stools and no fever. Mucus and blood are characteristic of stools from patients. This syndrome can be treated with the amebicidal drugs.

Toxoplasmosis: This disease is caused by *Toxoplasma gondii*. It is obligate intracellular parasite.

Domestic and wild cats are the only definitive hosts for the intestinal or sexual phase of this organism. Pigs are the major animal food source to humans.

Sarcocystosis: Sarcocystosis caused by *Sarcocystis hominis*, *S. suis*, when humans ingest a sarcocyst, bradyzoites are released and penetrate the lamina propria of the small intestine. Symptoms are stomachache, diarrhea, nausea.

Cryptosporidiosis:

Cryptosporidiosis caused by *Cryptosporidium parvum* source of contamination from faecal matter. It causes diarrhea.

Cyclosporiasis:

Cyclosporiasis caused by *Cyclospora cayentanensis*. Symptoms include diarrhea, loss of appetite, fatigue and weight loss.

LECTURE-10

Food borne animal parasites-Flat worms-Fascioliasis, Fasciolopsiasis, Paragonimiasis, Clonorchiasis, Diphyllbothriasis, Taeniasis, Round worms (Trichinosis, Anisakiasis)-Types of food involved - toxicity and symptoms-Chemical properties- Environmental conditions.

Flat worms

Fascioliasis: It is caused by *Fasciola hepatica* organism exists where sheep and cattle are raised.

Fascioliasis in cattle and sheep is a serious economic problem that results in the condemnation of livers. Humans are infected when raw or improperly cooked food taken.

Fasciolopsiasis: It is caused by *Fasciolopsis buski*. This parasite occurs in the duodenum and jejunum of humans and pigs where certain uncooked aquatic plants are consumed.

Paragonimiasis: This parasitic disease also known as parasitic hemoptysis is caused by *Paragonium westermani*. Symptoms include chronic coughing and sharp chest pains. Sputum is often reddish brown or bloody.

Clonorchiasis: *Clonorchis sinensis* commonly infect liver, lungs or blood of mammals.

Diphyllbothriasis: Infection occurs when raw or undercooked fish is consumed. Causative organism is *Diphyllbothrium latum*. It is often referred to as the broad fish tapeworm. The definitive hosts for *D.latum* are humans and other fish eating mammals. When human faces are deposited in waters, the eggs hatch and release six hooked, free swimming larvae. When these forms invade small crustaceans, they metamorphose in to a juvenile stage called Metacestode or procercoid larvae. When a fish ingests the crustacean, the larvae migrate in to its muscle and develop in to plerocercoid larvae. Humans are infected when they eat fish containing these forms. Symptoms include epigastric pain, abdominal cramps, vomiting, loss of appetite, dizziness and weight loss. Diphyllbothriasis can be prevented in humans by avoiding the consumption of raw or undercooked fish. Cooking fish products to an internal temperature of 60°C for 1 minute or 65°C for 30 seconds will destroy the organism.

Cysticercosis/ Taeniasis: Caused by *Taenia saginata* (beef tape) *Taenia solium* (pork type). When under cooked beef or eaten, these are infected.

Round worms: 1. Trichinosis: *Trichinella spiralis* is the etiological agent of trichinosis or trichinellosis. Trichinosis caused when flesh consumed infected with trichinae larvae.

2. Anisakiasis: This round worm infection caused by *Anisakis simplex* (herring worm or whole worm) and *Pseudoferranova decipiens* (cod worm or seal worm). Infection occurs by consumption of under cooked fish.

Lecture-11

Food borne viruses-Polio, Hepatitis A & E, Noro viruses, rota viruses, Prion Diseases- Types of food involved - toxicity and symptoms-Chemical properties- Environmental conditions.

Food Borne Viruses

In recent years viruses have been increasingly recognized as an important cause of food borne illness.

Polio: The Enterovirus is made up of small (28nm), single stranded RNA viruses and includes poliovirus, polio can be a transient viraemia with an incubation period of 3-5 days and characterized by headache, fever and sore throat. In later stages it invades central nervous system.

Contaminated milk had been the principal source of food borne polio. Polio is now virtually eradicated.

Hepatitis A & E: Hepatitis A is the cause of infections hepatitis. Hepatitis A is transmitted by the faecal- oral route. Milk, fruits like strawberries and raspberries, vegetables lettuce and shellfish are common food vehicles.

Hepatitis E – is non A, non B hepatitis virus. It is a calici like virus. It is also transmitted by the faecal – oral route and produces illness after an incubation period of 40 days.

Symptoms caused by this virus include anorexia, fever, malaise, nausea and vomiting, followed after a few days by symptoms of liver damage such as the passage of dark urine and Jaundice.

Noroviruses: Norwalk, nor walk like and small round structured viruses (SRSV) have been placed in this group. This virus was first recognized in a school outbreak in Norwalk, Ohio in 1968 and was suspected. Virus is more resistant to destruction by chloride than other enteric viruses. Gastroenteritis caused by their virus.

Rota viruses: The first demonstration of these viruses occurred in 1973 in Australia. Six groups have been identified. Group A is the most commonly encountered among infants and young children. Group b causes diarrhea in adults and they have been seen only in China.

They belong to family Reoviridae, they are about 70 nm in diameter, non enveloped and contain double stranded RNA. Faecal – oral route is the primary mode of transmission.

Prion Diseases: Prion are unique proteins in that they can convert other proteins into damaging ones by causing them to alter their shape. These particles were named around 1982 by Stanley Prusiner. (Nobel prize 1997). Scrapie in sheep, goats and hamsters Kuru in humans.

Creutzfeldt – Jakob disease (CJD) in humans. Bovine Spongiform disease encephalopathy (BSE) in cattle and sheep referred to as "mad cow disease".

LECTURE-12

Metals as toxins- Heavy metals- Arsenic-Occurrence – detection in foods- Toxicological effects- limits -Cadmium-Occurrence – detection in foods- Toxicological effects- limits - Mercury-Occurrence – detection in foods- Toxicological effects- limits.

Heavy metals are elements having atomic weight between 63.546 and 200.590 and a specific gravity greater than 4.0

Living organisms require trace amounts of some heavy metals; including Cobalt (Co), iron (Fe), copper (Cu), Manganese (Mn), Molybdenum (Mo), vanadium (V), Strontium (Sr) and Zinc (Zn). They are essential to maintain the metabolism of the human body.

Non essential metals of surface water systems are Cadmium (Cd), chromium (Cr), Mercury, (Hg) lead (Pb), Arsenic (As) and Antimony (Sb).

Food can become contaminated with toxic metals due to the following reasons.

- 1) Accidental mixing of food with metallic compounds such as arsenic oxides, Barium carbonate, Lead arsenate and others.
- 2) The material of the food container being dissolved by the may be cooked or stored in the vessel
- 3) Treatment of the food with metallic pesticides or excess food additives to prevent infection and spoilage as well as to secure special effects.
- 4) The presence of some metals naturally in the food Ex: Marine foods.
- 5) High ambient air concentrations near emission sources.

Arsenic:

Most important compounds of arsenic are arsenic (III) oxide (As_2O_3), yellow sulfide orpiment (As_2S_3), red realgar (As_4S_4), Paris Green or Copper (II) acetoarsenite $\{\text{Cu}(\text{C}_2\text{H}_3\text{O}_2) \cdot 3\text{Cu}(\text{AsO}_2)_2\}$. Calcium Arsenate ($\text{Ca}_3(\text{AsO}_4)_2$) and lead hydrogen Arsenate (PbHAsO_4).

Copper II acetoarsenite, Calcium arsenate and lead hydrogen arsenate are used as agricultural insecticides and poisons.

Orpiment and realgar formerly used as painting pigments.

Inorganic arsenic and its compounds, upon entering the food chain. Metabolized to a less toxic form of arsenic through a process of methylation.

Arsenic contamination of ground water has caused a massive epidemic of arsenic poisoning in Bangladesh and neighbouring countries.

World Health organization stands of As 10ppb. Arsenic in the ground water is of natural origin and is released from the sediment in to the ground water due to the anoxic conditions of the subsurface.

Most foods contain less than 0.2µg/g

Fish contain 1-10µg/g

Bottom feeders and shellfish 100µg/g

Lipid and water soluble organo arsenic compounds have been found but water soluble organ arsenic present in larger portion.

Arsenic absorption occurs predominantly from ingestion from the small intestine. Arsenic exerts its toxicity by inactivating up to 200 enzymes.

Mode of Action:

1. Arsenic exerts its toxicity by inactivating up to 200 enzymes especially those involved in cellular energy pathways and DNA Synthesis and repair.
2. Arsenic affects sulfhydryl groups of various enzymes such as pyruvate dehydrogenase and α -keto glutarate dehydrogenase.
3. British Anti Lewisite (BAL) was developed as antidote for arsenic poisoning. Use of BAL is limited because it causes some adverse effects such as fever, conjunctivitis, lacrimation headache, nausea and pain at the injection site.
4. Acute arsenic poisoning is associated initially with nausea, muscular weakness, vomiting, brown pigmentation, localized edema and severe diarrhea.
5. Acute toxicities by experimental animals are LD₅₀ (oral) of As⁵⁺ and As³⁺ are 1000mg/kg and 5mg/kg respectively.
6. Chronic arsenic toxicity results in multisystem disease. Chronic symptoms of arsenic poisoning are liver enlargement, anemia and reduction of white blood cells.
7. Bladder cancer would occur when 10ppb of arsenic consumed for a prolonged time period.

Mercury:

Mercury occurs primarily in geographical belts in the earth's crust as red sulfide, cinnabar. Earlier days Hg used in medicines to remedy bowel obstructions but now it is not used in remedies in modern medicine. Metallic mercury salts are used primarily by the electrical and chemical industries in switches, coatings and catalysts. Organo mercurial compounds are used to some extent as diuretics, but are being increasingly replaced by non mercurial compounds. Also organomercurials in various forms are very popular antiseptics and are used, in some cases in

sterilizing solutions for medical instruments. Mercury continues to be used in the dental preparations for fillings.

Metallic mercury deposited in a lake settles into the sediment where bacteria may carry out oxidations and alkylations producing both water soluble substance and lipid soluble substances.

Ex: Highly toxic organo mercurial compound used primarily as an algicide and will eventually be converted to methyl mercury, the most toxic of the mercurial compounds.

Mercury levels of most plant foods and meats are generally considered to be quite low.

Mercury levels in metals in the United States are in the range of 1-7ppb.

Potatoes, legumes and cereals < 50 ppb.

Methyl mercury accounts for greater percentage of mercury compounds in meat products and fish.

Inorganic mercury to be the major form of mercury in plant foods.

Fish is the primary source of dietary mercury.

Large marine fish have higher concentrations of mercury than smaller fish.

FDA – maximum of acceptable levels of Hg- 0.5ppm in fish and shell fish

WHO – maximum acceptable level of Hg in foods other than fish is 0.05ppm

WHO - suggested a provisional tolerable intake of Hg of 0.3mg total/week? This is not to include more than 0.2mg of methyl mercury.

Toxicity:

Less than 0.01% of ingested metallic mercury is absorbed from the gastrointestinal tract. Approximately 80% of inhaled metallic mercury in the vapour state is absorbed in the respiratory tract.

After absorption methyl mercury moves to the plasma, where it is bound in the red blood cells. It moves to the kidneys, colon, muscles and other tissues.

Toxic effects of mercury compounds in humans have been known for many years. Inorganic mercury affects primarily the kidneys leads to uremia and anuria.

The early symptoms of acute inorganic mercury poisoning are gastrointestinal upset, abdominal pain, nausea, vomiting and bloody diarrhea.

Acute mercury poisoning occurred in 1954 in Japan also called as Minamata disease because the poisoning occurred in Minamata Bay.

Minamata disease symptoms include loss of sensations at the extremities of the fingers, toes and areas around the mouth, loss of coordination, slurred speech, diminution of vision called tunnel vision and loss of hearing.

Pregnant woman exposed to methyl mercury gave birth to infants with mental retardation and cerebral palsy.

Cadmium

Cadmium has chemical characteristics similar to those of zinc. Cadmium occurs in nature in the wherever Zn is found and produced as a byproduct in the mining of

Zinc and lead. It is used in the galvanizing of other metals to prevent rusting and in the manufacture of storage batteries and plastics.

Ultimate source of contamination of these products is difficult to discover. Cadmium in water results from the cadmium alloys used to galvanize water pipes.

Major sources of cadmium exposure for the general population are water, food and tobacco.

Food generally contains less than 0.05ppm of cadmium, providing estimated 0.5mg cadmium per week.

WHO indicate that the foods with the highest levels of cadmium contamination are consistently shellfish and the kidneys of various animals including cattle, chicken, pigs, sheep and turkeys.

Cadmium levels in foods.

Kidneys - >10ppm

Shell fish -200-300ppm

Meats - 0.03ppm

Hamburger -0.075ppm

Soya beans – 0.09ppm

Apples -0.003ppm

Provisional tolerable intake set by WHO are 57-71 µg/ day.

Toxicity:

Cadmium is absorbed in the intestinal tract. Absorbed cadmium distributed to the liver, spleen, adrenals and cadmium. Cadmium poisoning (epidemic) occurred in the Jintau River valley of Japan. Kindly damage and skeletal disorders accompanied by great pain is observed. The disease was called Itai- Itail Byo or ouch – ouch disease.

Although the metal is resistant to alkalies, it is readily dissolved by acids present in fruit juices, wines, soft drinks, tomatoes etc. The salts of cadmium thus formed dissolve in the food and can produce in about 15-30 minutes after ingestion of the contaminated food, increased secretion of saliva, headache, vomiting, and persistent diarrhea abdominal cramps and acute gastritis. Later the

liver and kidneys may be affected; a dose of 10mg of the metal is enough to produce marked poisoning. Zinc has been shown to have a detoxifying protective effect against cadmium intoxication.

Metal Elimination steps:

1. Avoid mercury-silver dental amalgams. Mercury based amalgams are the major source of mercury in most Americans.
2. Avoid first and some shell fish: Fish and sea food are a common source for mercury and even arsenic especially the largest fish (shark, sword fish, tuna) which are highest in mercury – free fish and sea food
3. Clean water: use a high quality water filtration unit for all drinking water A high quality shower filter also helps to avoid inhalation of heavy metals and other contaminations
4. Clean Air: outdoor Air pollution is hard to avoid, but indoor air pollution is often much worse due to our energy inefficient, air tight buildings. High quality air filtration and purification systems have quickly become an important part of any healthy life style.
5. Clean Food: Buying organic foods or extensive washing of the surface of fruits and vegetables is a very important step as well as avoiding virtually all processed foods.
6. Natural products: Household cleaners as well as many personal care products such as skin creams and makeup have been found to carry heavy metal and other toxic elements by all natural products wherever possible.
7. Thimerosal free vaccines: Thimerosal has not been removed from all vaccines. Thimerosal is a mercury based preservative.
8. Avoid Industrial Areas : As much as possible avoid areas of industry especially in choosing where to live Air, Water and Food contamination is typically worse in these areas.

LECTURE-13

Lead, Tin, Zinc, Aluminium, Chromium, Cobalt, Antimony-Occurrence – detection in foods-Toxicological effects- limits

Lead

Lead occurs widely throughout the environment and found in all bodies of water and soils tested.

Lead occurring chiefly as sulfide galena in the earth's crust.

The principal causes of environmental lead contamination in recent times is it's in lead storage batteries and gasoline antiknock additives (tetra ethyl lead).

Gasoline contains 2-4g of lead per gallon.

Lead is exhausted out the tailpipe as particulate from gasoline.

Major non industrial sources of lead contamination for people are food and water.

U.S public Health service maximum allowed limit in drinking is 50mg/lit. WHO standard limit in drinking water is 0.01ppm. Natural level of lead to be on the order of 0.3ppb in marine fish according to recent analyses. These fish are considered good indicators of general environmental conditions.

Plant foods grown in industrialized areas exhibit higher lead levels compare to foods grow in remote areas.

Mode of toxic Action:

- 1) Organic lead compounds such as tetra ethyl lead are readily absorbed from the gastrointestinal tract and concentrating primarily in the bone, liver, kidney, muscle and central nervous system.

Absorption of inorganic lead in infants and children is considerably higher.

3 Stages of lead poisoning have been recognized.

- 1) Asymptomatic stage – generally not associated with behavioral disorders or organ disfunction, but is characterized by changes in the blood. Anemia is a well established early symptom.
- 2) Symptomatic stage: Anemia, disorders of the central nervous system including hyperactivity, impulsive behaviour, perceptual disorders and slowed learning ability, in more severe cases, symptoms include restless ness, irritability, atoxia and loss of memory.
- 3) In stage III, kidney failure, convulsion, coma and death is observed.

Milk has been recommended as a good antidote for lead poisoning.

The provisional to liberate weekly intake of lead as laid down by the FAO/WHO joint expert committee is only 3mg per person (or) 0.05mg/kg body weight.

Tin

Many foods come into close contact with tin during their preparation and storage. Canned food stuffs and beverages normally contain tin in amounts which are usually below 100mg/kg. Major source of tin contamination of foods is tin plate, which is a mild or low carbon steel sheet or strip coated with tin to make it rust proof.

A variety of containers are with tin plate for storing oils, fats, beverages, jams, fruits, vegetables, biscuits, confectionary coffee, tea and all types of processed foods. Conditions which favour excessive internal detinning of the containers are the following.

- 1) When the canned food is acidic.
- 2) When the food contains nitrates, oxalates, triethyl amine oxide, anthocyanin pigments, various dyestuffs added to carbonated beverages, copper (or) certain sulphur compounds.
- 3) When moist food is left in the can, after opening.

It is desirable to reduce tin contamination of canned foods to a minimum by making the cans from a twice- lacquered tin plate and later giving the interiors of the can a final sprayed coating. The lacquer or enamel used for the purpose must be non –toxic free from odour and flavor: resistant to the canned food stuff. The lacquers used are generally based on resins such as vinyl or epoxy resins. In some cases, even the lacquered cans are not safe from corrosion. For example stone fruits such as cherries and plums are canned without removing the stones; there will be an unexpected severe corrosion of the lacquered can. This is due to an enzyme present in the stone, which accelerates the corrosion. If the heat processes used in canning are sufficient to inactivate the enzyme, however there is no need to remove the stones from the fruits.

Hazards of tin:

When occasionally consumed in minute quantities tin does not seem to adversely affect the human system.

The toxic dose in food which causes acute intoxication is believed to be around 2-3gm. This dose causes severe headache, vomiting, photophobia, abdominal pain, dehydration and retention of urine.

Zinc:

This metal is present in some drinking waters and in the soil, thus gaining access to various foods such as cereals, milk, eggs, fish, vegetables, meat etc.

Continued ingestion of small quantities of the metal seems to have no harmful effect on man in fact, it is essential for good health. Its toxicity is manifested only when high concentrations are taken in single doses.

The longer the period of the storage, the greater will be the amount of zinc dissolved by the food. Even so these amounts are constitutively smaller than the amounts taken up by the food during cooking.

Acidic foods cooked in zinc vessels usually cause zinc poisoning. The symptoms are as usual dizziness, vomiting, colic and diarrhea.

Antimony:

Food poisoning due to antimony has been rare. When it did happen, it was usually mild and of short duration. The antimony being dissolved out from cheap antimony enamelware by acid foods such as lemonade and vinegar.

Wine and beer conveyed through rubber tubings were also found to be contaminated with the metal. Antimony toxicity observed if it is absorbed in higher concentrations. Symptoms include dryness of the throat, pain in swallowing, persistent nausea, occasional vomiting, loss of appetite and weight.

Aluminium

It is an established practice in North India to decorate sweet meats, on the top with silver foil. But instead of silver, very often, thin aluminium foil is used which means instead of the inert silver the reactive aluminium metal is consumed. Metallic aluminium can cause undesirable reactions in the digestive tract and interfere with its normal functioning.

Aluminium salts such as alum used in relatively large amounts as a constituent of baking powders of self raising flours, have been shown to interfere with growth and reproductive functions, in experimental animals. In man, alum baking powders have been found to cause gastric troubles. However FDA rules permit the use of acid compounds of aluminium in baking powder.

Chromium:

This metal in its trivalent form is required in traces by human body for optimal growth and long life. But when it is present it is acutely toxic even in very small quantities.

The practice of polishing whole turmeric with the bright yellow lead chromate is therefore, fraught with serious consequences, since the chromium in chromate is hexavalent. It itself is a dangerous. In fact, lead chromate is one of the two most toxic lead salts.

Cobalt: Cobalt, in traces is considered essential for good health. In fact, it is the most powerful stimulus for the formation of blood. But when ingested in large amounts, it becomes toxic.

Many cases of cardiopathy, some of them fatal, have been reported as a result of drinking beer containing the metal.

Metal Elimination steps:

1. Avoid mercury-silver dental amalgams. Mercury based amalgams are the major source of mercury in most Americans.
2. Avoid first and some shell fish: Fish and sea food are a common source for mercury and even arsenic especially the largest fish (shark, sword fish, tuna) which are highest in mercury – free fish and sea food
3. Clean water: use a high quality water filtration unit for all drinking water A high quality shower filter also helps to avoid inhalation of heavy methods chalse and other contaminations
4. Clean Air: outdoor Air pollution is hard to avoid, but indoor air pollution is often much work due to our energy efficient, air light buildings. High quality air filtration and purification systems have quickly become an important part of any healthy life style.
5. Clean Food: Buying organic foods or extensive washing of the surface of fruits and vegetables is a very important step as well as avoiding virtually all processed foods.
6. Natural products: House hold cleaners as well as many personal care products such as skin creams and makeup have been found to carry heavy metal and other toxic elements by all natural products where ever possible.
7. Thimerosal free vaccines: Thimerosal has not been removed from all vaccines. Thimerosal is mercury based preservative.
8. Avoid Industrial Areas : As much as possible avoid areas of industry especially in choosing where to live Air, Water and Food contamination is typically worse in these areas.

LECTURE-14

Pesticides-Chlorinated pesticides and non chlorinated pesticides-Decontamination of food commodities of their insecticide residues.Movement of Residues in the environment.

Definition: A pesticide is defined as any substance intended for preventing, destroying, attracting, repelling or controlling pests including unwanted species of plant or animals during the production, storage, transpiration, distribution and processing of the food, agricultural commodities or animal's feed or which may be administered to the animals for the control of ectoparasites.

According to FAO (1986) pesticide is defined as

Any substance or mixture of substances intended for use as plant growth regulator (PGR), defoliant or dessicant. The term excludes fertilizers and antibiotics or other chemicals administered to animals to stimulate their growth or to modify their reproductive behaviour.

Types of Pesticides:

- 1) Chlorinated pesticides
- 2) Non chlorinated pesticides

1) Chlorinated pesticides

1. DDT, 2. BHC, 3. Chlordane, 4. Toxaphene, 5. Aldrin, Dieldrin, 6. Methoxychlor, 7. Heptachlor 8. Mirex, 9. Dicofol, 10. Endosulfan, 11. Telodrin, 12. Chlordecone (Kepone)

2. Non chlorinated Insecticides

1) Carbamates:

Dome ton, pyrolan, Isolan, Carbaryl, propoxur (Baygon), Mexacarbate (Zectran), Aminocarb, Mathiocarb, Metacil, prome carb (Carbamult), Aldicarb, Aldoxycarb (Aldicarb sulfone), Landrin, Dioxacarb, Primicarb, Bendiocarb (Ficam) Indoxacarb, Alanycarb, Furathio carb, Carbosulfan and Fenoxycarb.

2) Organo phosphates

Organo phosphates are the most versatile chemical group, which has been explored to synthesize insecticides, fungicides, herbicides, nematocides and chemosterilants.

These insecticides were highly effective and thus required in relatively less amounts per unit area, and did not suffer from high persistence.

Therefore these toxicants rapidly replaced use of chlorinated pesticides.

3 groups of organophosphates (Ops)

1. Aliphatic Ops
2. Phenyl Ops
3. Heterocyclic (Ops)

1. Aliphatic Ops:

These are carbon chain like structures. The first Op used in Agriculture, TEPP

Ex: Schadan, TEEP, Dime fox, Malathion, Demeton, Mevinphos, Phorate, Dichlororos, Ethion, Alephate, Monocrotophos, Dicrotophos etc.

2. Phenyl Ops:

The Phenyl Ops contain a phenyl ring one of the ring hydrogens replaced by attachment to the phosphorous moiety and other hydrogens replaced by Cl, NO₂, CH₃, CN or S.

The phenyl Ops are generally more stable than the aliphatic.

First phenyl Op brought to agricultural use was ethyl parathion.

Ex: Parathion, methyl Parathion, profenophos, Dichlorophos, Fenamiphos, Tetra chlorvinphos, Iodofephos, crotoxyphos, phenthoate, Fenthion chlorfenviphos, Fenitrothion.

3) Hetero cyclic Ops:

This group contains heterocyclic ring in their structure.

Ex: Coumaphos, Diazinon, Azinphos, morphothion, phosalone, Methidathion, Chlorpyriphos, Quinalphos, Triazophos, Primiphos.

4) Pyrethroids:

Natural pyrethrum (pyrethrins) used for agril purpose.

Ex: Dimethrin, Tetramethrin

5) Neonicotiroids:

These insecticides have systemic action and kills flea on cats and dogs

Ex: Imidacloprid, Acetamiprid, Fiproles or (phenyl pyrazoles), Benzoyl ureas, Fungicides, Herbicides, Nematicides,

Algicides: Control algae in lakes, canals, swimming pools, water tanks and other sites.

Antifouling agents: kill or repel organisms that attach to under water surfaces, such as boat bottoms.

Antimicrobials: kill microorganisms

Fungicides: kill fungi

Disinfectants and Sanitizers: kill or inactive disease – producing microorganisms on in animate objects.

Fumigants: produce gas or vapour intended to destroy pests in buildings or soil.

Herbicides: kill weeds and other plants that grow where they are not wanted.

Insecticides: kill insects and other arthropods.

Miticides: Also called as (Acaricides)

Molluscides: kill snails and slugs.

Nematicides: kill nematodes

Ovicides: kill eggs of insects and mites.

Pheromones: Biochemicals used to disrupt the mating behaviour of insects.

Repellents: Repel pests

Rodenticides: control mice and other rodents.

Defoliants: cause leaves or other foliage to drop from a plant.

Global Scenario:

Global generic market is Rs 45000 crores.

Indian Scenario:

- India is the 4th largest producer of Agrochemical after U.S.A, Japan and China
- India is the second largest producer of pesticides in Asia
- India exports pesticides for approximately Rs 2800 crores.
- Total agrochemical market in India Rs 4500 crores.

Decontamination of food commodities of their Insecticide Residues

Washing removes extraneous insecticide residues from fruits and vegetables up to 20-100% depending upon several factors.

Blanching accomplished in either steam or hot water removes 50-73% of the residues including DDT.

Peeling of fruits and vegetables removes 90% of absorbed pesticide contaminants.

Home preparation and cooking of many food products were found to possess high potential in degrading even heat stable insecticides.

a) Vegetables and fruits: Washing and cooking processes removes pesticidal residues from contaminated vegetables obtained from the insecticide treated crops.

Dipping the vegetables in 2% salt solution for 10 minutes followed by water wash removes residues treated with bifenthrin.

Decontamination of groups treated with pesticides can be done by dipping in 59.9 -69.9% solution of tamarind, salt, acetic acid solutions.

Decontamination of groups treated with acephate, Monocrotophos, Quinalphos, dichlorvos, carbonyl can be done by dipping in 1% common salt vinegar for 10 minutes.

b) Wheat flour: Wheat flour was decontaminated during bread making to the extent of 50%

c) Milk and Milk products:

Pasteurization of milk at 12°C for 15 seconds has been found to degrade DDT, DDE, DDD, carbonyl and fenvalerate.

High temperature and reduced pressure have been used for decontamination of milk from heptachlor successfully to the extent of 95-100%. U.V irradiation of thin film of milk and ghee could reduce their loads of DDT and Methoxychlor.

d) Edible oils:

Most organic pesticides have an affinity towards oils which makes their removal extremely difficult. 100% removal of DDT from mustard oil by steam distillation. Alkali treatments and adsorbents (Fuller's earth and activated carbon) were found comparatively less effective.

e) Water:

Zero valent iron (Fe^0) has been found to promote reductive dechlorination and nitro group reduction of a wide range of pesticides in water. It is found that $\text{Al}_2(\text{SO}_4)_3$ added to water synergistic to the zero valent iron in enhancing pesticide degradation.

Sources of Insecticide Residues in Agricultural Commodities

There are four major ways which insecticides may reach agricultural commodities and leave their residues in/ on them, as follows:

1. Purposeful foliar or soil application of insecticides in food and forage crops for insect pest control.
2. Absorption of insecticides of by crop plants from soils reaching there through (a) run –off from other treated areas, (b) incorporation of stubbles of insecticide treated crops of previous season, and (c) use of irrigation water from either insecticide treated bodies of water or from the ones polluted with pesticide factory discharges.
3. Absorption of persistent insecticides from polluted environment and spray and vapour drifts of insecticides used for public health reasons.
4. Abuses of insecticide like
 - (I) their mixing with stored food stored food grains,
 - (II) Pre- marketing treatment of vegetables and fruits with insecticides to improve their appearance, and
 - (III) Disposal of left over insecticide treated seeds later as food grain for human consumption.
5. Injudicious use of insecticides

There can also other venues of insecticides reaching the agricultural commodities in specific situations.

Sources of Insecticide contamination of Food

Although food materials (eg milk, fish, eggs, honey, meat, and processed food) are not normally directly treated with insecticides yet, frequently, these are found contaminated with insecticides, some of which are not even in current use in agriculture. Many a time these residues are in concentrations above the officially acceptable Maximum Residue Limits (MRLs).

The major sources of contamination of food with insecticides are as follows:

1. In bovine milk and milk products the basic source of insecticide contamination is the insecticide treated feed and fodder fed to the milk animals. There could be other sources of contamination like their direct exposure to
 - A. persistent insecticides from the atmosphere and
 - B. Spray and volatile drifts from crop fields and areas treated for the control of human disease vectors. The OC insecticides like DDT and HCH are well known to stay in the atmosphere for months and years from where these can get absorbed into the exposed milk and milk products.
2. Insecticide residues in mothers' milk have been traced to their contaminated daily diet.
3. In honey the insecticides may find their way through nectar collected by honey bees from flowers of the insecticide- treated plants.
4. Poultry may consume grains from insecticide treated fields, which may finally land up in their meat and eggs.
5. Fish and other food absorb most of the insecticides from their surrounding water which may receive these (insecticides) from the
 - (i) Treated deep water paddies, (II) accidental slips: (III) pesticide factory discharges, and (IV) intentional introduction in fishery waters to stupefy fish for their easy catches.
6. In meat the major source of insecticide contaminations is the insecticide treated forages and concentrates consumed by the animals. It is reported that propensity of animal tissues to store pesticides, particularly in their fat, may be characteristic, irrespective of the amount ingested through their feed.
7. Drinking water can receive insecticides as contaminants through leaching from the treated soils, diffusion from polluted air into surface water bodies, polluted rain fall, etc.

8. Application of insecticides in homes, hotels and food stores for the control of household pests can be important sources for the control of household pests can be important sources of contamination of processed food of all kinds.

Factors in persistence of Insecticides

Although the persistence of insecticides in / on treated plants is desirable from the point of view of protracted pest suppression yet, their continuance as residue burden in edible parts of agricultural commodities at time of their harvest and more essentially at the time of their consumption, is unwanted for the health of the consumer.

The persistence of insecticides in plants is governed by three major factors.

(A) Transfer: physical removal of any insecticide after its application to plants by either rain or as volatile drift from the treated plant surfaces into the atmosphere is called its transfer. The rain-washing of insecticides is often more from erect plants with narrow, upright leaves than from the spreading ones. Also, it is more rapid from the waxy leaf surfaces than from the hairy leaves. Maturity level and density of plants at the time of pesticide spray are also important in this respect. Since in nature there exist numerous combinations of these plant features, the ease of washing down of a pesticide from plants on to the soil below, will vary widely within individuals of the plant kingdom. Besides, the time interval between the intensity of initial rain fall, will affect pesticide washing capability of the rain fall. Further the nature of the adjuvants used by the formulators of insecticides and their extent of systemic action can modify the pattern of rain- wash losses. The ultra-low-volume (ULV) sprays of pesticides are generally less prone to washing by rain than the conventional high volume sprays (HV).

Volatile drifts of insecticides from the foliage of treated crops from another important source of their transfer. It is depended upon vapor pressure of the pesticide molecule on one hand and the prevailing weather conditions (mainly temperature and RH) on the other. High vapor pressures of chemicals lead to their fast volatilization.

(B) Metabolism: The course of metabolism of insecticides within plants is probably a primary controller of their persistence in them. The basic molecular structures of insecticides, besides some other features, are dominant in this respect. For instance the organochlorinated (OC) insecticides are highly persistent in living tissues of plants and animals while the organophosphates, carbamates, and synthetic pyrethroids are much less so and, therefore, offer better choices for use in agricultural commodities. One may, however, note that metabolism of applied insecticides in plants does not

always lead to their reduced persistence. A few insecticides may actually become more persistent upon metabolism, e.g. when aldrin is metabolized to dieldrin and heptachlor to heptachlor epoxide.

(C) Crop Growth Dilution: When insecticides are applied early in the crop season, there is usually, there is usually adequate opportunity for them to get diluted by the time crop grows and matures and is for harvest. But the trouble arises crop grows and matures and is ready for harvest. But the trouble arises when insecticides are applied, contrary to recommendations, close to the crop harvest time, probably to ensure complete freedom of crops from pests which in fact, may not be necessary. This is usually the with many vegetable and fruit crops.

How to Minimize Insecticide Residues?

With the growing awareness of different kinds of environment pollution and health consciousness at global level, the pollution originating from pesticide residues in foodstuffs (and feeds) has been recognized as of paramount importance. The scientists have therefore been rightly concerned for quite some time with the development of means to minimize these residues to acceptable levels and discover methods to decontaminate certain foodstuffs of their pesticide residues. In these two very important efforts, joint cooperation of farmers, pesticide manufacturers, scientists, and consumers of the food are very essential.

Presently there are three main strategies available to minimize or limit insecticide residues in Agricultural Commodities and food, as follows:

1. Research and development of consumer-safe, alternative insect pest management technologies.
2. Regulation of proper use of synthetic insecticides.
3. Integrated Pest Management (IPM).

Chlorinated pesticides:

These are also called as Chlorinated hydrocarbons or organochlorines. These compounds are characterized by (A) the presence of carbon, chlorine, hydrogen and some times oxygen atoms, including a number of C-Cl bonds

(B) The presence of cyclic carbon chains. (Including benzene rings).

(C) Lack of any particular active intramolecular sites.

(D) A polarity and lipophilicity.

(E) Chemical unreactivity (i.e., they are stable in the environment)

These chemicals become concentrated in the food chain. Once deposited in the fat of mammals including humans, chlorinated hydrocarbons remain there indefinitely.

Major categories of these chemicals include the chlorinated bicyclodienes (or cyclodienes), hexachlorocyclohexanes, toxaphene and the DDT group. The cyclodienes include aldrin, dieldrin, chlordane, heptachlor, endrin and endosulfan. Hexachlorocyclohexanes is lindane (γ -BHC). Toxaphene is a complex mixture of chlorination isomers that also possess the bicyclic cage-like structure of the cyclodienes. These chemicals are often considered to belong to the group of organochlorine pesticides. However, their group characteristics make them very different from other organochlorine pesticides, such as fumigants, chlorinated organophosphates and chlorinated aliphatic and aromatic acids.

Non chlorinated pesticides

They include organophosphates, carbamates, Bipyridilium Herbicides, thiocyanate Insecticides, phenols, Fluorocarbonyl derivatives, Acaricidal chemicals, Fumigants, Inorganic insecticides, Botanical insecticides, synergists and microbial Insecticides.

They are not persistent, so they are not concentrated in the food chain.

Movement of Residues in the Environment

Major source of environmental contamination by pesticides are the deposits resulting from application of these chemicals to control agricultural pests.

Pesticides are dispersed mainly by water and air movements and are picked up by various biological systems. At the same time they are chemically or biochemically transformed to other nontoxic or toxic compounds in the environment.

Biological movement can include uptake and translocation of some pesticides by plants as well as bioaccumulation through food chains.

The movement of pesticides residues invariably depends on specific molecular structure and associated physical properties. For example, the more water soluble pesticides tend to dissolve in water and move very easily in the water.

The less soluble types tend to bind to soil or vegetation, thus being physically less mobile. Hydrophobicity also provides some potential for a chemical to accumulate in lipid reservoirs such as adipose tissue in animals or oils and oily products of plants.

LECTURE-15

Pesticides-Mechanisms of Toxicity-Residues in Food, Acceptable daily intakes, Maximum residue limits.

Mechanism of Toxicity

The use of pesticides in agricultural production represents three related but distinct risks defined as the quantifiable probability that harm or injury will occur.

1. The environmental risks associated with adverse effects on nontarget organisms and ground water contamination.
2. Occupational risks to agricultural workers and pesticide factory workers, which are considerably higher than those of other sources of human exposure and pose the foremost human health concern related to pesticides.
3. The occurrence of pesticides as residues on or in edible foods.

Chlorinated hydrocarbons have become most hazardous to human health through a process called **biomagnification**. Spray drifts and drainage from treated fields contaminate water bodies. Small plankton and other organisms living in the water absorb the pesticides and store them in tissue. The next animal feeding on the plankton in the food chain takes in a diet enriched with the pesticides and their metabolites. After a period the concentration of these residues rises in the animals. There is thus step wise increase in pesticide concentration along the food chain eventually. They all accumulate in human adipose tissue with increasing age. This property is often expressed as a **Bioconcentration Factor** (BCF) reported for hydrophobic chemicals having a tendency to partition from the water column and bioconcentrate in aquatic animals.

$$\text{BCF} = \frac{\text{Conc. Of the Chemical in the organism at equilibrium}}{\text{Conc. Of the Chemical in water}}$$

→ The inhalation route usually is the most rapid mechanism of uptake and most direct route to the blood stream.

→ High water soluble pesticides enter dermally.

Liver:

Liver plays a central role in the ability of an animal to deal effectively with both acute and chronic exposure to toxic chemicals.

Liver enlargement occurs by wide range of insecticides including pyrethrum, aldrin, dieldrin, chlordane, endrin, mirex, Chlordane. Liver enlargement also occurs in most vertebrate species.

Liver weight induction by chronic exposures to insecticides is most often associated with proliferation of smooth endoplasmic reticulum (SER) and mixed function oxidase induction. There is a critical difference in the mode of action of carbamates and organophosphates. For carbamates: the esterase inhibition is readily reversible. For organophosphates it is more permanent. Organophosphorus pesticides exhibit a special kind of neurotoxicity, which appears unrelated to acetyl cholinesterase inhibition. In this case, the neurotoxic signs appear several hours or days after exposure or ingestion and thus, the condition has been termed **delayed neurotoxicity**.

Delayed neurotoxicity is characterized by irreversible demyelination of nerves in the many species of mammals, including humans. Major group of organophosphates that can cause delayed neurotoxicity are the phenyl phosphonothioates.

Behavioral effects:

Mammalian behavior is extremely sensitive to xenobiotic chemicals. Very low exposure levels can cause decreased learning and memory function, hyper activity, altered aggressive and defensive behavior or abnormal courtship, resulting indirectly in reduced reproduction.

Ex: Toxaphene, endrin, Dieldrin, permethrin, Deltamethrin.

Immuno suppression:

Pesticides may suppress the immune competence of the host, animals become more susceptible to infection.

Ex: Dioxin, methyl parathion, carbofuran, hexachloro benzene, trichlorfon, molinate, DDT.

The physiological and biochemical effects of chronic exposure of vertebrates to pesticides have included increases in protein and lipid synthesis, changes in carbohydrate metabolism, hepatic glycogen depletion, changes in hepatic soluble enzymes (gluconeogenic enzymes) and induction of the microsomal mixed function oxidases (MFOs)

Common pathological findings in Livers.

1. Cellular hypertrophy
2. Fatty degeneration
3. Cellular margination
4. Increased basophils
5. Increased fat granules
6. Cytoplasmic vacuolization
7. Necrosis
8. Increase in smooth endoplasmic reticulum (SER)

9. Atypical mitochondria
10. Scattered swollen degenerated hepatocytes
11. Enlargement of nuclei
12. Hypertrophy of nuclei

Kidney:

The mammalian kidney is a dynamic and complex organ. Excretion of wastes is a primary function, but the kidney also plays a significant role in the regulation of total body homeostasis.

Nervous System:

Both Organophosphorus and carbamate pesticides inhibit acetyl cholinesterase, an enzyme that regulates neurotransmission by hydrolyzing acetyl choline that accumulates at the synaptic junction during propagation of nerve impulse.

Allergenicity:

Allergic reactions also can develop in individuals or the adjuvants contained in the formulated products.

Estrogenicity:

Estrogenicity caused by DDT, Methoxychlor, kepone.

Estrogens may be viewed toxicologically from two points i.e., as tumor initiators and as tumor promoters are considered as syncarcinogens.

Carcinogenicity:

Majority of pesticides suspected to be carcinogenic belong to the chlorinated hydrocarbon group.

Mutagenicity:

Mutagenic effects represent the result of genetic impairments caused by introduced chemicals.

Ex: Carbamates, Ziram, Carbaryl.

Teratogenicity:

Effects on development of an embryo or fetus caused by maternal exposure have been demonstrated for a wide range of pesticidal chemicals.

Ex: Mirex, DDT, 2, 4, 5-T herbicide 2, 3, 7, 8- Tetra chloro di benzodioxin (TCDD).

Organophosphates – Dimethoate, Monocrotophos Methyl parathion. Mercurial fungicides.

Residues in Food

A national survey conducted by food marketing Institute (1988) showed that approximately 75% of consumers are very concerned about pesticide residues in their food.

- Presence of Ethylene dibromide and chlordane in foods leads to acute food poisoning.

- Aldicarb contamination of watermelon.
- Several countries have established national monitoring programs for a wide range of pesticides.

Information on food contamination can be obtained through two different approaches
Regulatory and commodity monitoring services and focus on raw agricultural commodities.

→ Virtually every food group is contaminated with residues. Of all the major classes of compounds, the chlorinated hydrocarbons are the most commonly found contaminants.

DDT and its degradation products and metabolites followed by dieldrin, hexachloro cyclohexone (BHC) and poly chlorinated biphenyls (PCBs).

→ DDT was previously found in almost all major classes of food. Now days it is banned. In USA it is banned in 1972. Particularly meats and dairy products reflect it's persistence in the adipose tissues of animals as well as pesticidal residues in mother milk is 10-30 times higher than that of cow's milk.

→ In milk most frequently occurring residue is 4-4'-dichlorophenyl – trichloro ethane (P, P'-DDE) and dieldrin. Residue measured was 0.02 ppm.

→ Population exposures to pesticides in the diet can be assessed by a Market basket total diet study conducted by FDA. In general residues present at or above **1 ppb** could be measured.

→ Malathion continues to be the residue most frequently found.

→ According to Market basket surveys fats and oils and the meat and poultry groups were most likely to contain pesticide residues.

→ Highest measured intake was for dieldrin.

Following are some key points that have become apparent from the extensive surveys on pesticide residues in humans.

1. The chemicals found to accumulate in humans are mostly chlorinated hydrocarbons. These are stable and lipophilic and are detectable at very low concentrations.
2. The levels of any given pesticide vary geographically and among various segments of the population
3. Changes in the levels of stable pesticides over time are rather slight.
4. The major factor determining the distribution of pesticides in the body is **fat content** however; there are a number of other factors influencing the final distribution of residues.

Acceptable Dairy Intakes: (ADI)

WHO and FAO has recommended ADIs of pesticide residues i.e., maximal daily intake of a chemical which, during a lifetime, appears to be without appreciable risk.

These values are updated at regular intervals. The judgement is based on toxicological, pharmacological and biochemical data on experimental animals and wherever available on humans. Actual estimates of the ADI are usually 1% of the no-effect level of the most sensitive animal species tested.

The ADI is also applicable to the main pesticide metabolites if those detected in food are the same as those in experimental animals.

MRL - Maximum Residue Limit

ERL - Extraneous Residue Limit

Pesticide	ADI mg/kg body weight	MRL or ERL (mg/kg body weight)
1) Butyl amine	0.1	-
2) Captan	0.1	0.1
3) Cartap	0.1	20
4) Chlordimeform	0.0001	2
5) Chlorothalonil	0.03	0.2
6) Coumaphos	0.0005	0.02
7) Lyanofenphos	0.005	-
8) DDT	0.005	0.05-5

LECTURE-16

Antinutrients-Toxic phenolic substances, Flavonoids, tannins, Coumarin- Toxicity and symptoms-Chemical properties (structure and stability) –Type of foods involved-Prevention.

Introduction

Our food contains, in addition to the many well-known major (protein, fat, carbohydrate, and fiber) and minor (vitamins, minerals, and nonessential compounds) nutrients, thousands of naturally present toxic plant compounds.

Many of these compounds are commonly termed “nature’s pesticides” because they are often toxic to predators, such as insects and animals.

Tannins

Tannins long have been known as plant materials. They confer a dark color when applied to animal hides thereby turning them into “tanned” leather. Tannins are a large group of water-soluble polyphenolic compounds with a molecular weight greater than 500 that have the ability to bind to and/or precipitate proteins. It is their ability to bind to proteins that is of toxicological and nutritional concern.

Tannins also strongly bind to metals, such as iron, copper, and zinc, and reduce the gastrointestinal absorption of these metals.

The two major classes of tannins are the **proanthocyanidins** (or “condensed tannins”) which are flavonoid polymers, and **hydrolyzable tannins**, which are polymers of gallic or ellagic acid esterified to either glucose or a polyphenol, such as catechin.

Tannins occur in nearly every plant-derived food, but they are particularly high in bananas, raisins, spinach, red wines, bracken fern, coffee, and tea. Tea is an especially rich source of tannins. Green tea has about 4%, while black tea may contain as much as 33% tannin; adding milk to tea will bind the tannins so that they will be less absorbable. A normal diet will provide several grams per week from fruits and vegetables.

Tannins also are high in traditional herbal stimulant drinks such as those derived from Brazilian guarana (*Paullinia cupana*), betel nut (*Areca catechu*), and kola nut (*Cola nitida* and *C. acuminata*).

Tannins inhibit dietary protein digestion. Tannins are liver carcinogens. Habitual chewers of betel nut (primarily in India, Pakistan, and Southeast Asia) have a high incidence of carcinoma of the mouth which has been linked to the high tannin content (10 to 25%) of this nut, although other components may be involved.

Some polyphenolic tannins are also anticarcinogenic. Other polyphenolic tannins are Caffeic Acid and Chlorogenic Acid.

Coumarin and Psoralen

Coumarin is widely found in plants such as cabbage, radish, and spinach, and in plants traditionally used as flavoring agents, such as lavender and sweet woodruff (*Asperula odorata*); the latter is an essential herb for making May wine, which is a popular German drink used to salute the coming of Spring. Coumarin is widely found in herb teas based on tonka beans (*Dipteryx odorata*) and sweet clover (*Melilotus albus* and *officinalis*) called “melilot.”

The name “coumarin” originates from *coumarou*, the Carribean name for tonka beans. Purified coumarin was once used as a food additive, but this use was banned by the FDA after it was discovered that high doses caused liver damage.

Psoralens are a group of phototoxic furocoumarins widespread in a number of plant families such as Apiaceae (formerly Umbelliferae — celery and parsnips), Rutaceae (e.g., bergamot, limes, cloves), and Moraceae (e.g., figs). When activated by sunlight, psoralens are mutagenic, presumably due to their ability to form interstrand and protein cross-links with DNA.

Miscellaneous Flavonoids: Quercetin, Ellagic Acid, Kaempferol, and Rutin

This family of chemicals is widespread in plant-derived foods, including fruits and fruit juices, vegetables, buckwheat, tea, cocoa, red wine, dill, soybeans, bracken fern, and others. The estimated average daily intake of flavonoids is 1 g. None of these has yet been conclusively shown to be carcinogenic, but both quercetin and kaempferol are mutagenic. Rutin is not mutagenic in itself, but it can be metabolized by intestinal bacteria to yield quercetin. Quercetin also has some anticarcinogenic properties.

Goitrogens: Goiter is defined as a noncancerous and non inflammatory enlargement of the thyroid gland and has been recognized since ancient times.

Favism: Favism is a hemolytic disorder and caused by exposing to pro oxidant chemicals found in fava beans (*Vicia faba*). Symptoms of favism include hemolytic anemia, jaundice and hyperbilirubinemia.

Neurolathyrism: Neurolathyrism is an ancient disease caused by consumption of peas of the genus *Lathyrus sativus* commonly known as vetch pea, chickling pea or grass pea. Neurolathyrism is known to occur in humans following consumption of at least 300g/day of the peas for a period of at least 3 months. Initial symptoms are increasing rigidity muscles and loss of control over the legs.

LECTURE-17

Antinutrients- Saffrole, Myristicin, Cyanogenic glycosides, Glucosinolates, Allyl isothiocyanates, Favism, Lathyrism, Canavanine, Acetylcholinesterase inhibitors, Goitrogens, Caffeic acid & Chlorogenic acid, Glycoalkaloids, Saponins.

Canavanine

Alfalfa sprouts contain up to 15,000 ppm canavanine. Canavanine is produced in other legumes as well, such as the jack bean. It is an analog of arginine and, as such, can substitute for this amino acid in cellular proteins, thereby compromising their function. Canavanine inhibits the enzyme nitric oxide synthetase and induces heat-shock proteins in human cells. Due to its action as an antimetabolite, it is under current consideration as an antitumor drug in combination with other antimetabolites such as 5-fluorouracil, but has not yet been tested for carcinogenicity. Canavanine is suspected of causing autoimmune disorders in people, such as lupus erythematosus. Primates fed alfalfa sprouts develop a severe toxic syndrome resembling human lupus.

Cyanogenic Glycosides

Cyanogenic glycosides are cyanide-containing compounds naturally present in seeds from apples, apricots, cherries, peaches, pears, plums, quinces, and also in almonds, sorghum, lima beans, cassava, corn, yams, chickpeas, cashew nuts, and kirsch. High cyanide varieties, distinguished by their bitter taste, may contain over 600 ppm cyanide on a dry weight basis, while “sweet” varieties contain much less. There are several such cyanogenic glycosides, of which linamarin, amygdalin, and dhurrin are examples.

Cyanogenic glycosides are toxic by virtue of the release of free hydrogen cyanide which occurs when the plant tissue is disturbed as during chopping, processing, or ingestion. These conditions initiate the hydrolysis of the glycoside by the action of β -glucuronidases and other enzymes naturally present in the plant tissue and in the intestinal lumen. Hydrolysis by β -glucuronidases produces the sugar and a cyanohydrin, the latter spontaneously or enzymatically degrades to form free hydrogen cyanide. Cyanide is one of the most acutely toxic chemicals. It binds to and inactivates heme enzymes, the most critical of which is mitochondrial cytochrome oxidase, resulting in an acute, life-threatening anoxia.

Cyanogenic glycosides in cassava probably represent the greatest health risk. Traditional methods of processing cassava, such as sun-drying, soaking, boiling, and fermenting, eliminate most of the cyanide. In addition to regular cases of human deaths, cyanogenic glycosides in cassava

may be responsible for birth defects, endemic goiter, and “konzo,” an upper myelopathic motor neuron disease.

Allyl Isothiocyanates

Allyl isothiocyanates are a group of major naturally occurring compounds that confer a pungent flavor to foods, such as mustard and horseradish, where it is present at about 50 to 100 ppm. It is also present at much lower levels in Brassica vegetables such as broccoli and cabbage, and in cassava and other tropical staple foods.

Isothiocyanates occur in cruciferous vegetables as glucosinolate conjugates that are hydrolyzed when the plant releases enzymes such as during chewing. Isothiocyanates are toxic goitrogens which inhibit binding of iodine in the thyroid gland. Because iodine is required for the formation of the critical thyroid hormones thyroxine (T₄) and triiodothyronine (T₃), isothiocyanate-induced hyperthyroidism (goiter) mimics iodine deficiency. Hyperthyroidism is a physiological response as the thyroid attempts to compensate for reductions in both T₄ and T₃ production.

Hydrazines and Other Toxins in Edible Mushrooms

The three most commonly eaten mushrooms are the cultivated mushroom (*Agaricus bisporus*), the shiitake mushroom (*Cortinellus shiitake*), and the false morel (*Gyromitra esculenta*). All contain substantial amounts of compounds in the hydrazine family many of which are potent liver toxins and animal carcinogens. N-methyl-N formyl hydrazine is commonly found in concentrations of 500 ppm and causes cancer.

Shiitake mushrooms and the cultivated mushroom contain up to 3000 ppm agaritine. A metabolic product of agaritine (a diazonium derivative) is a potent carcinogen and a mutagen. Gyromitrin (acetaldehyde-N-methyl-Nformylhydrazone) the major carcinogenic hydrazine in the false morel, also is present in similar concentrations.

Another carcinogenic hydrazine is methylhydrazine is present in smaller concentrations (14 ppm). A diet of whole *A. bisporus* mushrooms (30% of total diet) did not cause a significant increase in tumors.

Toxic Substances in Spices and Flavoring Agents

Safrole, estragole, myristicin, β -asarone, piperine, and isosafrole are closely related alkenylbenzenes found in many spices, essential oils, and herbs. They also are present, in much lower levels, in parsnips, parsley, and sesame seeds. All are weak to moderate rodent hepatocarcinogens.

Safrole:

Safrole is found in sassafras tea and makes up 85% of oil of sassafras (*Sassafras albidum*), which was once used to flavor root beer. It has been banned as a flavor additive since 1960, but is a minor, natural component of nutmeg, mace, star anise, cinnamon, and black pepper. Sassafras bark is an ingredient in file powder used to make gumbo, a spicy Cajun dish.

Estragole

Estragole, a related aromatic flavor agent, is found in tarragon, basil, and fennel, and is likewise a weak carcinogen.

Isosafrole, a component of ylang-ylang (*Cananga odorata*) oil, a flavorant and scent, is carcinogenic in mice.

β -Asarone is a major component of oil of calamus (derived from the *Acorus calamus* root which is a folk remedy for indigestion), and was once used to flavor vermouth and bitters. It causes intestinal tumors in rats.

Myristicin

It is a major flavor component of nutmeg, derived from the dried, ripe seed of the tree *Myristica fragrans*. Approximately 2% of nutmeg is myristicin, which is present in the volatile oil distilled with steam from the dried seeds. Mace, a closely related spice, is derived from the arilode, or outer coating of the seed. Myristicin is found in black pepper, parsley, celery, dill, and carrots as well. It is carcinogenic, large amounts of nutmeg, equivalent to two whole nutmeg seeds (ca. 15 g) are intoxicating and allegedly hallucinogenic. Large doses also are associated with undesirable side effects, such as tachycardia, flushed skin, and dry mouth.

Piperine, an alkaloid present in high concentrations (10%) in black pepper (*Piper nigrum* and other sp.), is largely responsible for the pungent “bite” of this condiment. Powdered *P. cubeba* berries are added to cigarettes and smoked as a remedy for throat irritation, and oil derived from these berries is added to some throat lozenges. Under appropriate conditions, piperine is chemically converted to potentially carcinogenic intermediates.

Capsaicin is the extremely pungent ingredient (up to about 0.5%) in red and yellow chili peppers: *Capsicum frutescens*, *C. conoides*, and *C. annum*. Due to its irritating qualities to the eyes and mucous membranes, a solution of capsaicin in an aerosol spray is a popular dog repellent for mail carriers. Topical creams containing capsaicin (0.025%) are commercially available as an analgesic. Capsaicin is a weak carcinogen.

Glycyrrhizin is a saponin-like glycoside derived from the dried roots of *Glycyrrhiza glabra*, popularly known as licorice. Licorice is one of the oldest folk medicines traditionally used as an expectorant, flavoring agent (also used to mask the bitter taste of medicines), and demulcent. Cuneiform tablets dating to about 4000 B.C. mention the medicinal use of licorice by the Sumerians, and pieces of licorice root was found in King Tut's tomb.

The one caveat to the many benefits of licorice is that it promotes hypertension.

Licorice reportedly has been responsible for fatal episodes of acute hypertension in people. Consequently, people with heart problems or hypertension should avoid licorice; as little as 100 to 200 g/day can cause persistent, heightened mineralocorticoid activity.

d-Limonene:

It is a major constituent of citrus oils and also found in other fruits and vegetables in much lower amounts. The major sources of d-limonene are oils of orange, grapefruit, and lemon. Citrus peel oil can contain as much as 95% d-limonene. d-Limonene per se or citrus oils where d-limonene is the major constituent have been widely used as flavoring agents and/or as fragrances in perfumes and soaps, and in a variety of foods such as ice cream, soft drinks, baked goods, gelatin, chewing gum, and puddings. It is also the active ingredient in "natural" citrus-based degreasing solvents and in insect repellents. d-Limonene is nephrotoxic.

Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids (PAs) are common plant toxins produced by over 200 species of flowering plants, from genera such as *Senecio*, *Crotalaria*, and *Cynoglossum*. They are often present at very high levels — as much as 5 % of the plant's dry weight. Pyrrolizidine alkaloid-containing plants pose significant health hazards to people who consume some kinds of "natural" herbal teas and traditional folk remedies and those who eat grain-based foods contaminated with PA-containing plant parts. These chemicals are often carcinogenic, mutagenic, and teratogenic and chronically hepatotoxic.

Petasitenine is found in *Petasites japonicus*, a medicinal herb used as an expectorant and cough suppressant. The flower stalks of this herb are used as a food or herbal remedy. When incorporated into the diet, dried stalks are hepatocarcinogenic.

Tussilago farfara (coltsfoot) is a common herb used for centuries as a medicine for coughs and bronchitis in Europe and Asia. (*Tussilago* is the ancient Roman name for "cough suppresser.") The plant contains the pyrrolizidine alkaloid senkirkine at concentrations as high as 150 ppm, as well as high concentrations of senecionine, another very toxic and carcinogenic PA.

Comfrey (*Symphytum officinale*) is a nearly universal herb commonly sold not only in health food stores and by herbalists, but also in supermarkets. Since ancient Greek and Roman times, both leaves and roots have been used to make teas and compress pastes to treat a variety of external and internal diseases, such as healing of wounds, skin disorders, and respiratory diseases. Numerous vegetarian recipes call for comfrey leaves to make soufflés, salads, and breads. Comfrey leaves and roots contain up to 0.3% pyrrolizidine alkaloids such as intermedine, lycopsamine, symphytine, and others. Diets containing powder from dried leaves and roots causes liver tumors.

Substances in Bracken Fern

Bracken fern (*Pteridium aquilinum*, *esculentum*, and others) is widely used as human food in greens or salads in many countries such as New Zealand, Australia, Canada, the U.S., and especially Japan. It is also a forage plant for sheep and cattle. It first attracted the attention of veterinary scientists who noticed severe toxicity — bladder cancer, bone marrow depression, severe leukemia, thrombocytopenia, and a hemorrhagic syndrome — in livestock grazing on this plant. Human consumption of bracken fern has been linked to an increased incidence of esophageal cancer in Japan. The major carcinogen in bracken is **ptaquiloside**, a potent norsesquiterpenoid glucoside that is present at often high concentrations (up to 1.3% dry weight) in the plant.

Acetylcholinesterase Inhibitors in Potatoes

Members of the family Solanaceae contain a variety of toxic glycoalkaloids. Potatoes (*Solanum tuberosum*) are an important food staple in many parts of the world and, under certain conditions, produce a variety of glycoalkaloids. Potatoes that have been damaged, exposed to light (green), or sprouted contain the glycoalkaloids α -solanine and α -chaconine that can exceed concentrations of 100 ppm. Higher amounts of solanine and chaconine are present in the potato greens (tops). Healthy potatoes contain negligible amounts of these toxins. Episodes of human poisoning by green potatoes have been documented. Poisoning symptoms — gastric pain, weakness, nausea, vomiting, labored breathing — are consistent with acetylcholinesterase inhibition.

Caffeic Acid and Chlorogenic Acid

Caffeic and its quinic acid conjugate chlorogenic acid occur in an extremely wide range of fruits and vegetables. Upon ingestion, chlorogenic acid is hydrolyzed in the gastrointestinal tract to yield caffeic and quinic acids. In humans, caffeic acid is metabolized to *o*-methylated derivatives, such as ferulic, dihydroferulic, and vanillic acids, and *meta*-hydroxyphenyl derivatives, which are excreted in the urine. Caffeic acid and conjugates are present in high concentrations (over 1500

ppm) in many seasonings (thyme, basil, anise, caraway, rosemary, tarragon, marjoram, sage, and dill); vegetables (lettuce, potatoes, radishes, and celery); and fruits (grapes, berries, eggplant, and tomatoes). Coffee is particularly rich in these phenolics, in addition to many other compounds (see below).

Acup of coffee contains about 190 mg of chlorogenic acid. Caffeic acid inhibits 5-lipoxygenase which is a key enzyme in the biosynthesis of various eicosanoids, such as leukotrienes and thromboxanes. These eicosanoids are mediators of a wide variety of physiological and disease states and are involved in immunoregulation, asthma, inflammation, and platelet aggregation. At high doses (2% in the diet), caffeic acid caused a significant incidence of forestomach squamous cell papillomas and carcinomas.

Miscellaneous Flavonoids: Quercetin, Ellagic Acid, Kaempferol, and Rutin

This family of chemicals is widespread in plant-derived foods, including fruits and fruit juices, vegetables, buckwheat, tea, cocoa, red wine, dill, soybeans, bracken fern, and others. The estimated average daily intake of flavonoids is 1 g. None of these has yet been conclusively shown to be carcinogenic, but both quercetin and kaempferol are mutagenic. Rutin is not mutagenic in itself, but it can be metabolized by intestinal bacteria to yield quercetin. Quercetin also has some anticarcinogenic properties.

Goitrogens: Goiter is defined as a noncancerous and non inflammatory enlargement of the thyroid gland and has been recognized since ancient times.

Favism: Favism is a hemolytic disorder and caused by exposing to pro oxidant chemicals found in fava beans (*Vicia faba*). Symptoms of favism include hemolytic anemia, jaundice and hyperbilirubinemia.

Neurolathyrism: Neurolathyrism is an ancient disease caused by consumption of peas of the genus *Lathyrus sativus* commonly known as vetch pea, chickling pea or grass pea. Neurolathyrism is known to occur in humans following consumption of atleast 300g/day of the peas for a period of atleast 3 months. Initial symptoms are increasing rigidity muscles and loss of control over the legs.

LECTURE-18

Anti nutritive factors-Type A antinutritives – antiproteins- Protease inhibitors- Lectins- Type B antinutritives – antiminerals – Phytic acid – Oxalic acid – Glucosinolates- Dietary fiber- Gossypol- Type C antinutritives – anti vitamins – Ascorbic acid oxidase – Antithiamine factors- Antipyridoxine factors.

I Type A antinutritives (antiproteins)

1. Protease inhibitors, 2. Lectins

II Type B antinutritives (antiminerals)

1. Phytic acid, 2. Oxalic acid, 3. Glucosinolates, 4. Dietary fiber, 5. Gossypol

III Type C antinutritives (antivitamins)

1. Ascorbic acid oxidase, 2. Antithiamine factors, 3. Antipyridoxine factors

Antinutritives can be of natural as well as synthetic origin. They can be classified as follows:

Type A: substances primarily interfering with the digestion of proteins or the absorption and utilization of amino acids; these are also called *antiproteins*;

Type B: substances interfering with the absorption or metabolic utilization of minerals (*antiminerals*);

Type C: substances that inactivate or destroy vitamins or otherwise increase the need for vitamins (*antivitamins*).

Antinutritives are mainly found in plant material. In a number of cases, drugs, antibiotics, and pesticides have been reported to be antinutritive. This chapter deals with food components of natural origin only.

Type A antinutritives (antiproteins)

Especially people depending on vegetables for their protein supply are in danger of impairment by antiproteins. This is often the case in less developed countries.

1 Protease inhibitors

Protease inhibitors are proteins which inhibit proteolytic enzymes by binding to the active sites of the enzymes. This category of antinutritives occurs in many plants, and in a few animal tissues. Proteolytic enzyme inhibitors were first found in eggs. They were later identified as ovomucoid and ovomucoprotein, both of which inactivate trypsin. Also, chymotrypsin inhibitors are found in eggs, especially in the egg white. Other foods in which trypsin and/or chymotrypsin inhibitors are found are legumes (e.g., soybeans), vegetables (e.g., alfalfa), milk, wheat and potato.

However, several protease inhibitors are relatively heat resistant. An example is the trypsin inhibitor in milk. In raw milk, the activity of trypsin can be reduced by 75 to 99%. The inhibitor is unaffected by temperatures up to 70°C. Other relatively heat-resistant protease inhibitors are the trypsin inhibitor in alfalfa, the chymotrypsin inhibitor in potatoes, and the trypsin inhibitor in lima beans.

2 Lectins

Lectins are the general term for plant proteins that have highly specific binding sites for carbohydrates. The majority of the lectins are glycoproteins. A carbohydrate-free lectin occurs in jack beans (concanavalin A). The lectin in kidney beans is probably a lipoprotein. The mode of action of lectins may be related to their ability to bind to specific cell receptors in a way comparable to that of antibodies. They can agglutinate red blood cells. Therefore, they are also called hemagglutinins. Lectins interfere with the absorption of amino acids, fats, vitamins, and thyroxine.

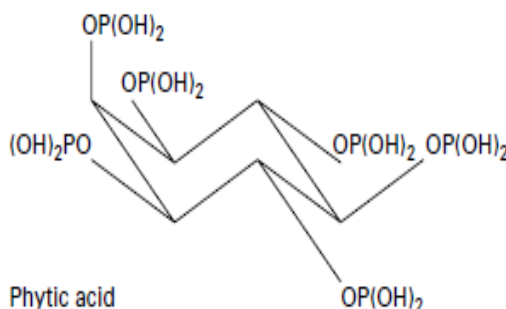
It will become clear that the lectins in legumes not only belong to type A antinutritives but also to types B and C. Lectins occur in plants, especially legumes such as peanut, soybean, lima, kidney, mung, jack, hyacinth, castor and fava bean, and lentil and pea. They are also found in potato, banana, mango, and wheat germ. Lectins can contribute to a large extent to the protein content of plants.

Type B antinutritives (antiminerals)

Substances interfering with the utilization of essential minerals are widely distributed among vegetables, fruits, and cereal grains. The levels of anti minerals in foods seldom cause acute effects.

1 Phytic acid

Phytic acid, the hexaphosphoric ester of myo-inositol, is a strong acid. It forms insoluble salts with many types of bivalent and tetravalent heavy metal ions. In that way, phytic acid reduces the availability of many minerals and essential trace elements.



Phytic acid shows negative effect on iron absorption in humans. The absorption of iron depends mainly on the levels in the iron pools, the amount and the chemical form ingested, and the presence of ascorbic acid. Ferric phytate is least soluble in diluted acid, i.e., it is insoluble in the stomach. At the pH in the duodenum, ferric phytate dissolves in the form of ferric hydroxide. Phytic acid prevents the complexation between iron and gastroferrium, an iron-binding protein secreted in the stomach. Results from animal experiments and human studies indicate interference of phytic acid with the absorption of magnesium, zinc, copper, and manganese.

2. Oxalic acid

Oxalic acid ($\text{HOOC}-\text{COOH}$) can induce toxic as well as antinutritive effects. To humans, it can be acutely toxic. However, it would require massive doses of 4 to 5 g to induce any toxic effect. The oxalic acid levels usually found in food are not much toxic.

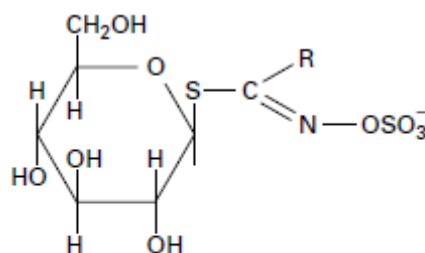
Like phytic acid, oxalic acid reduces the availability of essential bivalent cations. Oxalic acid is a strong acid and, with alkaline earth metal ions and other divalent metalions, it forms salts that are hardly soluble in water.

Calcium oxalate is insoluble in water at neutral or alkaline pH, and dissolves easily in an acid medium. In many animal experiments and human studies, negative effects of oxalate-rich foods have been found, especially on calcium absorption. Vegetable foods such as rhubarb, spinach, and celery, as well as cocoa have been shown to disturb the calcium balance in man.

3 Glucosinolates

A variety of plants contain a third group of antiminerals called glucosinolates, a class of thioglucosides, whose general structure is shown below.

Many glucosinolates are goitrogenic. Three types of goiter are distinguished: cabbage goiter (struma), brassica seed goiter, and legume goiter.



Glucosinolate

a. Cabbage goiter can be induced by the excessive consumption of cabbage. It seems that cabbage goitrogens inhibit iodine uptake by directly affecting the thyroid gland. Cabbage goiter can be treated by iodine supplementation.

b. Brassica seed goiter can result from the consumption of the seeds of Brassica plants, such as rutabaga (swede), turnip, cabbage, rape, and mustard, which contain substances that prevent thyroxine synthesis. This type of goiter can only be treated by administration of the thyroid hormone.

c. Legume goiter is induced by goitrogens in legumes such as soybeans and peanuts. It differs from cabbage goiter in that the thyroid gland is not involved directly. Inhibition of the intestinal absorption of iodine or the reabsorption of thyroxine has been shown in this case. Legume goiter can be treated by iodine therapy.

4. Dietary fiber

Dietary fiber is a collective term for all food components derived from plant cell walls that are not digested by the endogenous secretions of the human digestive tract. It has no clearly defined composition. It may differ from foodstuff to foodstuff, and from diet to diet. Dietary fiber consists of pectic substances, hemicelluloses, plant gums and mucilages, algal polysaccharides, celluloses, and lignin. Further, tannins, indigestible proteins, plant pigments, waxes, siliceous materials, and phytic acid can be incorporated in the fiber matrix. These materials give bulk to the fecal matter, not only from their inherent mass, but also by their water-binding capacity. The amount of water bound can be four to six times the dry weight of the fiber.

Dietary fiber has the capacity to bind various metals, even if the phytic acid is removed. Disturbed Ca^{2+} , Mg^{2+} , Zn^{2+} and P balances have been observed in human subjects using diets rich in fiber in the form of whole wheat bread. Secondly, amino acids and proteins are bound to dietary fiber. A diet containing 15% cellulose can cause a decrease in nitrogen absorption of as much as 8%. Carrageenans, which are highly indigestible, can cause a decrease in nitrogen absorption of about 16%. The interaction of dietary fiber with sugars does not result in a reduction of sugar absorption, but in a slow release of sugars into the bloodstream.

5. Gossypol

Gossypol is a plant antinutritive factor present in oil and protein that gained importance as a dietary oil and protein source, especially in tropical and subtropical countries. This antinutritive is a yellow pigment present in all parts of the cotton plant. The highest levels are found in cotton seed. Gossypol exists in three tautomeric forms: phenolic quinoid tautomer (I), aldehyde (II), and

hemiacetal (III). Gossypol is an anti mineral as well as an anti protein. It forms insoluble chelates with many essential metals, such as iron, and binds to amino acid moieties in proteins, especially to lysine. The protein binding suggests that gossypol can reduce the availability of food proteins and inactivate important enzymes. Processing removes 80 to 99% of the gossypol. The pigment is extracted with the oil and subsequently removed by refining and bleaching.

Type C antinutritives (antivitamins)

Anti vitamins are groups of naturally occurring substances which can decompose vitamins, form unabsorbable complexes with them or interfere with their digestive or metabolic utilization. Only the more relevant examples of this type of antinutritives are ascorbic acid oxidase, antithiamine factors, and anti pyridoxine factors.

1 Ascorbic acid oxidase

Ascorbic acid oxidase is a copper-containing enzyme that mediates the oxidation of free ascorbic acid first to dehydroascorbic acid and next to diketogulonic acid, oxalic acid, and other oxidation products. Ascorbic acid oxidase occurs in many fruits and vegetables such as cucumbers, pumpkins, lettuce, cress, peaches, bananas, tomatoes, potatoes, carrots, and green beans.

Its activity varies with the type of fruit or vegetable. The enzyme is active between pH 4 and 7. Its optimum temperature is about 38°C. When plant cells are disrupted the compartmentalization of substrate and enzyme is removed. Therefore, if vegetables and fruits are cut, the vitamin C content decreases gradually. In fresh juices, 50% of the vitamin C content is lost in less than one hour. Being an enzyme, ascorbic acid oxidase can be inhibited effectively by blanching of fruits and vegetables. Ascorbic acid can also be protected against ascorbic acid oxidase by substances of plant origin. Flavonoids, such as the flavonoles, quercetin and kempferol, present in vegetables and fruits, strongly inhibit the enzyme.

2 Antithiamine factors

A second group of anti vitamins is the anti thiamine factors. They interact with vitamin B₁, also known as thiamine. Anti thiamine factors can be distinguished as thiaminases, tannins, and catechols. The interaction with vitamin B₁ can lead to serious neurotoxic effects as a result of vitamin B₁ deficiency. Normally, anti thiamine factors pose no appreciable risk to humans. They only cause thiamine deficiency in people whose diet is already low in thiamine.

Thiaminases are found in many fish species, freshwater as well as saltwater species, and in certain species of crab and clam. These antithiamine factors are enzymes that split thiamine at the methylene linkage

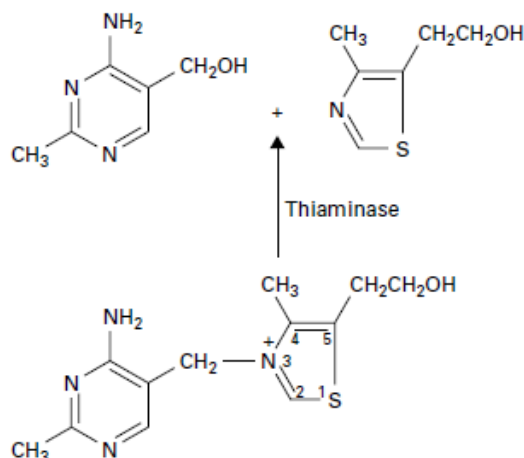


Figure 3.3 Degradation of thiamine by thiaminase.

Thiaminases contain a non protein coenzyme, structurally related to hemin, the red pigment component of hemoglobin. The coenzyme is the actual antithiamine factor. Cooking destroys thiaminases in fish and other sources.

3. Antipyridoxine factors:

A variety of plants and mushrooms contain pyridoxine (a form of vitamin B₆) antagonists. The antipyridoxine factors have been identified as hydrazine derivatives. Linseed contains the water soluble and heat-labile antipyridoxine factor linatine.

Linatine is γ -glutamyl-1-amino-D-proline. It readily undergoes hydrolysis to the hydrazine derivative, 1-aminoproline, the actual antipyridoxine factor. Antipyridoxine factors have also been found in wild mushrooms, the common commercial edible mushroom, and the Japanese mushroom shiitake. Commercial and shiitake mushrooms contain agaritine.

Agaritine is hydrolyzed in the mushroom by γ -glutamyltransferase to the active agent 4-hydroxymethylphenylhydrazine. The hydrolysis of agaritine is accelerated if the cells of the mushrooms are disrupted. Careful handling of the mushrooms and immediate blanching after cleaning and cutting can prevent hydrolysis.

LECTURE-19

Anti microbial agents –common anti microbial food agents – Benzoic acid – Benzoates- Sorbic acid – Sorbates – Short chain organic acids – acetic acid – lactic acid – propionic acid – citric acid – parabens –sulfite- nitrite.

Benzoates:

Sodium salt of benzoic acid has been used extensively as an antimicrobial agent in foods. It is incorporated into jams, jellies, margarine, carbonated beverages, fruits salads, pickles, relishes, fruit salads, pickles, relishes, fruit juices sodium benzoate is relatively ineffective at pH values near neutrality and the effectiveness increases with increase in acidity. Optimum pH for sodium benzoate is 2.5 – 4.0.

Two esters of p – hydroxy benzoic acid, methyl paraben and propyl paraben are also used extensively in foods. Butyl and ethyl esters are also used but limited use. These compounds are similar to benzoic acid in their effectiveness. They are effective at higher pH because the esterification of the carboxyl group.

Sorbic acid: Sorbates (sorbic acid as the calcium, sodium or potassium salt) used as a spray, dip or coating on packaging materials. It is widely used in cheeses, cheese products, baked goods, beverages, syrups, fruit juices, jellies, jams, fruit cocktails, dried fruits, pickles.

Sorbic acid and its salts are known to inhibit yeast and molds but are less effective against bacteria. They are effective at low P^H Values. ($pH < 6.0$)

Parabens:

Methyl and propyl parabens are permissible in foods up to 0.1%. Heptyl paraben is permitted in beers to a maximum of 12 ppm and up to 20 ppm in fruits drinks and beverages. They are effective at pH values up to 8.0.

Acetates:

Monochloro acetic acid, paracetic, dehydroacetic acid and sodium diacetate have been recommended as preservatives but not all are approved by FDA.

Dehydroacetic acid has been used to impregnate wrappers for cheese to inhibit the growth of molds, and as a temporary preservative for squash. Acetic acid in the form of vinegar is used in mayonnaise, pickles, catsup, pickled sausages and pigs test. Acetic acid is more effective against yeasts and bacteria than molds. Its effectiveness increases with decrease in pH.

Sodium diacetate has been used in cheese spreads and malt syrups and as treatment for wrappers used on butter.

Nitrites and Nitrates:

Combinations of these various salts have been used in curing solutions and curing mixtures for meats. Nitrites decompose to nitric acid which forms nitrosomyoglobin when reacts with the pigments in meats and thereby forms a stable red colour.

Nitrites can react with secondary and tertiary amines to form nitrosamines, which are known to be carcinogenic. Nitrites are currently added in the form of sodium nitrite potassium nitrite, sodium nitrate and potassium nitrate.

Inhibits *C. botulinum* in meat products.

Sulfur dioxide and sulfites:

Egyptians and Romans burned sulphur to form sulphur dioxide a means of sanitizing wine making equipment and storage vessels.

Sulphur dioxide and sulphites are used in the wine industry to sanitize equipment and to reduce the normal flora of the grape must.

They form sulphurous acid and effectiveness is enhanced at low P^H . Affects microbial cells by reduction of disulfide linkages, formation of carbonyl compounds, reaction with ketone groups and inhibition of respiratory mechanisms.

Fumes of burning sulphur are used to treat most light colored dehydrated fruits. SO_2 also used in syrups, fruit juices and wine making. In addition to antimicrobial action of sulfites, they are also used to prevent enzymatic and non enzymatic changes or discoloration in some foods.

Propionic Acid:

Propionates – (sodium or calcium propionate) are used most extensively in the prevention of mold growth and rope development in backed goods and for mold inhibition in many cheese foods and spreads. Also used in butter jams, jellies, figs, apple slices and malt extract.

They are effective against molds, little effect on most yeast and bacteria. Their effectiveness decreases with an increase in P^H . optimum activity at P^H 5-6. They are ideal preservatives for bread and baked goods. Propionic acid is a short chain fatty acid ($CH_3 CH_2 COOH$) affects cell membrane permeability. Propionic acid is found naturally in Swiss cheese up to 1%.

Acetic, propionic, and sorbic acids are short-chain fatty acids used primarily as preservatives.

LECTURE-20

Anti microbial agents - Natural antimicrobial substances present in foods(Indirect antimicrobials)-Antioxidants, Flavoring agents, spices and essential Oils, phosphates, Medium chain fatty acids and esters, acetic and lactic acid.

Natural antimicrobial substances present in foods (Indirect antimicrobials):

Antioxidants: Phenolic antioxidants possesses antimicrobial activity against a wide range of microorganisms, including some viruses, mycoplasmas, and protozoa.

Butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), and t-Butylhydroxyquinoline (TBHQ) are inhibitory to Gram-positive and Gram-negative bacteria, as well as to yeasts and molds at concentrations ranging from about 10-1,000 ppm, depending on substrate.

Flavouring agents:

Of the many agents used to impart aromas and flavors to foods, some possess definite antimicrobial effects. In general, flavor compounds tend to be more antifungal than antibacterial.

The nonlactic, Gram-positive bacteria are the most sensitive, and the lactic acid bacteria are resistant.

Some GRAS (Generally Recognised as Safe) indirect antimicrobial chemicals used in foods are:

- 1. Diacetyl:** One of the most effective flavoring agents is diacetyl, which imparts the aroma of butter. It is somewhat unique in being more effective against Gram-negative bacteria and fungi than against Grampositive bacteria.
- 2. 2, 3-Pentanedione:** Another flavor compound that imparts the aroma of butter is 2, 3-pentanedione, and it has been found to be inhibitory to a limited number of Gram-positive bacteria and fungi at 500 ppm or less.
- 3. l-carvone:** The agent l-carvone imparts spearmint like aromas and the agent d-carvone imparts caraway like aromas, and both are antimicrobial, with the l-isomer being more effective than the d-isomer; both are more effective against fungi than bacteria. Minimal inhibitory concentration is (MIC) 1,000 ppm or less.
- 4. Phenylacetaldehyde:** Phenylacetaldehyde imparts a hyacinth like aroma and has been shown to be inhibitory to *Staphylococcus aureus* at 100 ppm; and *Candida albicans* at 500 ppm.
- 5. Menthol:** Menthol, which imparts a peppermint like aroma, was found to inhibit *S. aureus*, *E. coli* and *C. albicans*
- 6. Vanillin and ethyl vanillin:** Vanillin and ethyl vanillin are inhibitory, especially to fungi.

Spices & Essential Oils: These originally present in the food or added purposely to prevent growth of micro organisms. Allicin – Garlic, onion, leeks. Phytoalexins are produced by many plants in response to microbial invasion. Eugenol – Allspice (pimento), cloves, cinnamon; Thymol – thyme and oregano. Cinnamic aldehyde – Cinnamon and Cassia. Inclusion of cinnamon in raisin bread retards mould spoilage.

Humulones contained in the hop resin and isomers produced during processing, impart the characteristic bitterness of beer.

Oleuropein – The bitter principle of green olives have antimicrobial properties.

Mustard oil inhibits Gram negative and positive bacteria and some yeasts.

Although used primarily as flavoring and seasoning agents in foods, many spices possess significant antimicrobial activity.

Phosphates: These salts are commonly added to certain processed meats to increase their water holding capacity. They also contribute to flavor, and they are antioxidative.

Food-grade phosphates range from one P (e.g. trisodium phosphate) to at least 13 (sodium polyphosphate). The microorganisms inhibited by phosphates are *Clostridium botulinum*, *Bacillus cereus*, *Listeria monocytogenes*.

Medium Chain Fatty Acids and Esters:

Medium-chain fatty acids are employed primarily as surface-active or emulsifying agents. The antimicrobial activity of the medium-chain fatty acids is best known from soaps, which are salts of fatty acids. Those most commonly employed are composed of 12-16 carbons. For saturated fatty acids, the most antimicrobial chain length is C₁₂ for monounsaturated (containing one double bond), C_{16:1}; and for polyunsaturated (containing more than one double bond), C_{18:2} is the most antimicrobial. In general, fatty acids are effective primarily against Gram-positive bacteria and yeasts. Although the C₁₂ to C₁₆ chain lengths are the most active against bacteria, the C₁₀ to C₁₂ are most active against yeasts. Saturated aliphatic acids effective against *C. botulinum*.

Monolaurin is the most effective of the glycerol monoesters, and sucrose dicaprylate is the most effective of the sucrose diesters. Monolaurin (lauricidin) are inhibitory to a variety of Gram-positive bacteria and some yeasts at 5-100 ppm.

Because the fatty acids and esters have a narrow range of effectiveness, and GRAS substances such as EDTA, citrate, and phenolic antioxidants also have limitations as antimicrobial agents when used alone.

Acetic, propionic, and sorbic acids are short-chain fatty acids used primarily as preservatives.

LECTURE-21

Anti microbial agents – Antibiotics (Monensin, natamycin, tetracyclins, Subtilin, Tylosin, Nisin, Endolysins,), Antifungal agents for fruits, Ethylene and propylene Oxides.

Antibiotics are secondary metabolites produced by microorganisms that inhibit or kill a wide spectrum of other microorganisms. Most of the useful ones are produced by molds and bacteria of the genus *Streptomyces*, and a few by *Bacillus* and *Paenibacillus* spp.

Two antibiotics have been investigated extensively as heat adjuncts for canned foods are subtilin and tylosin.

Chlortetracycline and oxytetracycline were once widely studied for their application to fresh foods, whereas natamycin is employed as a food fungistat.

Some considerations on the use of antibiotics as food preservatives were noted by Ingram et al., and several of the key ones are summarized as follows:

1. The antibiotic agent should kill, not inhibit, the flora and should ideally decompose into innocuous products or be destroyed on cooking for products that require cooking.
2. The antibiotic should not be inactivated by food components or products of microbial metabolism.
3. The antibiotic should not readily stimulate the appearance of resistant strains.
4. The antibiotic should not be used in foods if used therapeutically or as an animal feed additive.

The tetracyclines are used both clinically and as feed additives, and tylosin is used in animal feeds and only in the treatment of some poultry diseases. Neither nisin nor subtilin is used medically or in animal feeds. Nisin is used in many countries but not subtilin.

Monensin:

This antibiotic was approved by the FDA as a cattle feed additive. It is used primarily to improve feed efficiency in ruminants. Its amino acid-sparing action has been demonstrated in fistulated cows. It inhibits Gram-positive bacteria, and thus its long-term use has the potential of shifting the gastrointestinal tract bacterial biota from one that is normally Gram positive to one that is more Gram negative.

Like nisin, monensin is an ionophore (destroys selective permeability of cell membranes), and the two agents compare favorably as feed additives.

Natamycin: This antibiotic (also known as pimaricin, tennecetin, and myprozine) is a polyene that is quite effective against yeasts and molds but not bacteria. Natamycin is the international nonproprietary name, as it was isolated from *Streptomyces natalensis*.

In granting the acceptance of natamycin as a food preservative, the joint Food and Agriculture Organization/the World Health Organization (FAO/WHO) Expert Committee took the following into consideration:

1. It does not affect bacteria,
2. It stimulates an unusually low level of resistance among fungi,
3. It is rarely involved in cross-resistance among other antifungal polyenes.
4. DNA transfer between fungi does not occur to the extent that it does with some bacteria.

It is not used as a feed additive. Natamycin is effective against both yeasts and molds. Natamycin appears to act in the same manner as other polyene antibiotics-by binding to membrane sterols and inducing distortion of selective membrane permeability.

Tetracyclines:

Chlortetracycline (CTC) and oxytetracycline (OTC) were approved by the FDA.

A level of 7 ppm to control bacterial spoilage in uncooked refrigerated poultry is used. CTC and OTC are effective in delaying bacterial spoilage of not only fish and seafoods but poultry, red meats, vegetables, raw milk, and other foods. CTC is generally more effective than OTC.

The surface treatment of refrigerated meats with 7-10 ppm typically results in shelf-life extensions of at least 3-5 days. The tetracyclines are both heat sensitive and storage labile in foods, and these factors were important in their initial acceptance for food use.

Subtilin:

It is structurally similar to nisin, although it is produced by some strains of *Bacillus subtilis*. Like nisin, it is effective against Gram-positive bacteria, is stable to acid, and possesses enough heat resistance to withstand destruction at 121°C for 30-60 minutes.

Subtilin is effective in canned foods at levels of 5-20 ppm in preventing the outgrowth of germinating endospores, and its site of action is the same as for nisin. Like nisin, it is used neither in the treatment of human or animal infections nor as a feed additive.

Tylosin:

This antibiotic is a nonpolyene macrolide. It is more inhibitory than nisin or subtilin. Unlike nisin, subtilin, and natamycin, tylosin is used in animal feeds and also to treat some diseases of poultry. As a macrolide, it is most effective against Gram-positive bacteria. It inhibits protein synthesis by associating with the 50S ribosomal subunit.

Antifungal agents for fruits:

Some compounds applied to fruits after harvest to control fungal spoilage:

- 1. Thiabendazole:** Applied to control fungal spoilage of apples, pears, citrus fruits, pineapple.
- 2. Benomyl:** It is applied uniformly over the entire surface of fruits at the concentrations of 0.5-1.0g/l. It can penetrate the surface of some vegetables. It is used to control crown rot and anthracnose of bananas and stem end rots of citrus fruits.
- 3. Biphenyl:** Applied to control spoilage of citrus fruits.
- 4. SO₂ fumigation:** To prevent the spread of Botrytis from grape to grape, SO₂ is employed for long term storage. It is applied shortly after harvest and about once a week thereafter.
- 5. Sodium- α -phenylphenate:** Applied to control fungal spoilage of apples, pears, citrus fruits, pineapple.

Ethylene and Propylene oxides:

Ethylene and propylene oxides, along with ethyl and methyl formate are used as antimicrobial agents. They have similar action. The oxides exist as gases and are employed as fumigants in the food industry. The oxides are applied to dried fruits, nuts, spices, and so forth, primarily as antifungal compounds.

Ethylene oxide is an alkylating agent. Its antimicrobial activity is due to the presence of labile H atoms, the unstable three-membered ring of ethylene oxide splits. The H atom attaches itself to the oxygen, forming a hydroxyl ethyl radical, CH₂CH₂OH, which attaches itself to the position in the organic molecule left vacant by the H atom. The hydroxyl ethyl group blocks reactive groups within microbial proteins, thus resulting in inhibition. Among the groups capable of supplying a labile H atom are -COOH, -NH₂, -SH, and O-OH.

Ethylene oxide appears to affect endospores of *C. botulinum* by alkylation of guanine and adenine components of spore DNA.

Ethylene oxide is used as a gaseous sterilant for flexible and semi rigid containers for packaging aseptically processed foods. With respect to its action on microorganisms, it is not much more effective against vegetative cells than against endospores.

LECTURE-22

Antimicrobial agents- Miscellaneous chemical preservatives-Chitosans, Dimethyl dicarbonate, Ethanol, Glucose oxidase, Polyamino acids. Bacteriophages as biocontrol agents, Hurdle concept.

Chitosans:

These are cationic polysaccharides made from chitin by acid or enzymatic hydrolysis. They are deacetylated derivatives of chitin. O-carbomethylated (O-CM) chitosan is water soluble and has a broader antimicrobial spectrum than some other preparations.

Chitosans vary widely in molecular size with some having molecular weights as low as 30 to over 1,000. These compounds appear to be more effective against Gram-positive than Gram-negative bacteria, and they are being investigated as antimicrobial compounds for use in packaging films (bioactive packaging). The polycationic chitosans bind to negatively charged bacterial cells and interfere with membrane and transport functions.

DimethylDicarbonate:

This compound is used as a yeast inhibitor in wine and in some fruit drinks at a level of 0.025%. Upon hydrolysis, it yields methanol and CO₂.

Ethanol:

This alcohol is present in flavoring extracts and effects preservation by virtue of its desiccant and denaturant properties. Ethanol vapors, produced by a vapor generator, can be produced within the headspace of a package, and the vapors have been shown to be effective against some bacteria and fungi. Ethanol has been shown to sensitize *L. monocytogenes* to low pH, organic acids, and osmotic stress. It appears that ethanol alters membrane permeability and thus makes cells more susceptible to certain other agents. Spores of *Bacillus subtilis* treated with either alkali or ethanol release their DPA. Dehydroacetic acid is used to preserve squash.

Diethylpyrocarbonate has been used in bottled wines and soft drinks as a yeast inhibitor. It decomposes to form ethanol and CO₂ by hydrolysis or alcoholysis (reaction with ethyl alcohol.)

Glucose Oxidase: This enzyme catalyzes the oxidation of glucose, in the presence of O₂, to gluconic acid and H₂O₂. The enzyme is produced by some molds, and the products of the reaction suppress the growth Gram-negative bacteria.

Polyamino Acids: At least two amino acid cationic polymers are inhibitory to a number of food borne bacteria and fungi, and to possess minimal toxicity to humans. They apparently act by

combining and interfering with the activity of microbial cell membranes. The two noted below are reported to have GRAS status.

Epsilon-polylysine is produced in Japan, and levels as low as 5 ppm is inhibitory to some Gram-positive bacteria. It is water soluble, effective over a wide pH range, and is degraded to lysine by proteases.

The other compound is ethyl-N-dodecanoyl-L-arginine HCl (produced in Spain). Its effectiveness against meat and poultry micro biota has been demonstrated at levels of 200ppm. In the body, it is metabolized to arginine.

Bacteriophages as biocontrol agents:

Lytic phages specific for given bacterial species and strains are effective in destroying their host cells. Bacteriophages destroy their host cells in meats, poultry.

Foodborne pathogens inhibited by phages are *Vibrio vulnificus*, *Campylobacter jejuni*, *Salmonella enteridis*.

Hurdle concept:

Multiple factors or techniques are employed to effect the control of microorganisms in foods. Barrier technology, combination preservation and combined methods are among some of the other descriptions of this concept and referred to hurdle technology. Also called as barrier technology.

Example: Germination of spores of *Clostridium botulinum* prevented by both intrinsic and extrinsic parameters like pH less than 4.6, water activity (a_w) is less than 0.94, NaCl of 10% or more and Sodium nitrite at the concentration of 120ppm, incubation temperature of $\leq 10^\circ\text{C}$.

Nisin

Nisin is produced by some strains of *Lactococcus lactis*. The first use of Nisin is used was by Hurst to prevent the spoilage of Swiss cheese by *Clostridium butyricum*. This compound is effective against Gram positive bacteria. It is ineffective against Gram-negative bacteria.

Some of desirable properties of Nisin are

1. It is nontoxic.
2. It is produced naturally by *Lactococcus lactis*.
3. It is heat stable and has excellent storage stability.
4. It is destroyed by digestive enzymes.
5. It does not contribute to off-flavors and off-odors.
6. It has a narrow spectrum of antimicrobial activity.

LECTURE-23

Sanitation- GMPs- Personal hygiene – Sanitizers – Sanitation principles- Sanitizing methods- Sanitation agents – Chlorocompounds- Iodocompounds – Bromcompounds- Acid and alkali compounds.

Soil that remains on food processing equipment after use is usually contaminated with microorganisms nourished by the nutrients of soil deposits. This contaminant provides a medium for microbial proliferation. A sanitary environment is obtained through removing soil deposits with subsequent destruction of residual microorganisms. There are different sanitizing agents and application methods.

The United States Environmental Protection Agency (EPA) has defined three categories of microbial treatments based on their general level of effectiveness.

A **sterilant** is an agent that destroys or eliminates all forms of microbial life.

Chemical sterilants include ethylene oxide, glutaraldehyde, and peroxyacetic acid.

Heat, both dry heat ovens and moist heat such as steam under pressure, or autoclaving is a sterilization process.

A **disinfectant** is an agent that kills infectious fungi and vegetative bacteria although not necessarily bacterial spores on inanimate surfaces.

Disinfection is a less lethal process than sterilization. General disinfectants are the major source of products used in households, swimming pools, and water purifiers.

A **sanitizer** is a substance that reduces, but not necessarily eliminates microbial contaminants on inanimate surfaces to levels that are considered to be safe from a public health standpoint. A sanitizer is effective in destroying vegetative cells.

Sanitizers are regulated by the EPA and require stringent laboratory test data and registration. They are categorized as

- a) no-rinse food-contact surface sanitizers
- b) non-food-contact surface sanitizers.

Food-contact sanitizers include sanitizing rinses for equipment, utensils, and containers used in dairy processing plants, food processing, and beverage plants, and eating and drinking establishments.

A **biocide** is a substance that provides microbial control of a process (fogging disinfection, disinfection of an aseptic line, or biofilm removal). These compounds are classified as oxidative sanitizer biocides (various halogens), hydrogen peroxide-based biocides (peracetic acid, peracids,

chlorine dioxide, and ozone), and surfactant-based biocides (acid anionic sulfonic acid, sulfonated fatty acids, and quaternary ammonium compounds). Others are chlorohexidine gluconate, phenolics, and aldehydes (glutaraldehyde and formaldehyde).

SANITIZING METHODS

1. Thermal

Thermal sanitizing is relatively inefficient because of the energy required. Its efficiency depends on the humidity, temperature required, and length of time a given temperature must be maintained.

Microorganisms can be destroyed with the correct temperature if the item is heated long enough and if the dispensing method and application design, as well as equipment and plant design, permit the heat to penetrate to all areas. Temperature should be measured with accurate thermometers located at the outlet pipes to ensure effective sanitizing. The two major sources for thermal sterilization are steam and hot water.

a. Steam

Sanitizing with steam is expensive and usually ineffective. Workers frequently mistake water vapor for steam; therefore, the temperature usually is not high enough to sterilize that which is being cleaned. If the surface that is being treated is highly contaminated, a cake may form on the organic residues and prevent sufficient heat penetration to kill the microbes. Experience in the industry has shown that steam is not amenable to continuous sanitizing of conveyors. Condensation from this operation and other steam applications has complicated cleaning operations.

b. Hot Water

Immersion of small components (i.e., knives, small parts, eating utensils, and small containers) into water heated to 80°C or higher is another thermal method of sterilization.

The microbicidal action is thought to be the denaturation of some of the protein molecules in the cell.

Hot water is an effective, nonselective sanitizing method for food-contact surfaces; however, spores may survive more than an hour at 100°C. Hot water is frequently used for plate heat exchangers and eating utensils. If water hardness exceeds 60 mg/L, water scale is frequently deposited on surfaces being sanitized unless the water is softened. Hot water is readily available and nontoxic. Sanitizing can be accomplished either by pumping the water through assembled equipment or by immersing equipment in the water.

Radiation

Radiation at a wavelength of approximately 2,500 Å in the form of ultraviolet light or high-energy cathode or gamma rays will destroy microorganisms. For example, ultraviolet light has been used in the form of low-pressure mercury vapor lamps to destroy microorganisms in hospitals and homes. UV activity appears to be pH and temperature independent and produces no taste or odor in treated water.

There are three different sources of ionizing radiation available for the treatment of food products. They are electron beam, (e-beam), gamma rays, and X-rays.

E-beam radiation has the shortest penetration range of approximately 7.5 cm, whereas gamma and X-rays can penetrate one or more meters. Bacterial resistance determines the lethal exposure time. Radiation does not penetrate well and its use as an antimicrobial agent should be restricted to microorganisms on surfaces, in the air, or in clear liquids.

Liquids that may be treated with UV light include: beverage plant water, brine solutions, vegetable product transfer water, cleaning-in-place rinse water, heating and cooling water, cheese curd ranch water, and wastewater effluents.

High Hydrostatic Pressure (HHP)

This technique is applied to foods, which can be liquid or solid, packaged or unpackaged, to high pressure (which varies depending upon application) usually for 5 minutes or less.

HHP can be used on many foods such as raw and cooked meats, fish and shellfish, fruit and vegetable products, cheeses, salads, dips, grains and grain products, and liquids including juices, sauces, and soups. The high pressure does not destroy the food, because it is applied evenly from all sides. Microorganisms living on the surface and in the interior of the food are inactivated.

Inactivation is accomplished by affecting the molecular structure of chemical compounds necessary for metabolic metabolism in the microorganisms. HHP is equally effective on molds, bacteria, viruses, and parasites, and has achieved some success in treating bacterial spores, which are resistant to many biocidal processing treatments.

Vacuum/Steam/Vacuum

A process has been developed that exposes solid food products to vacuum, steam, and vacuum again. Although the process has not been fully explored at this time, it appears to have potential for the destruction of pathogenic microorganisms in fresh meat and poultry, processed meats, seafood, and fruits and vegetables.

Chemical Sanitizing

The chemical sanitizers available for use in food processing and foodservice operations vary in chemical composition and activity, depending on conditions. Generally, the more concentrated a sanitizer, the more rapid and effective its action. The individual characteristics of each chemical sanitizer must be known and understood.

Most appropriate sanitizer for a specific sanitizer application can be selected. Because chemical sanitizers lack penetration ability, microorganisms present in cracks, crevices, pockets, and in mineral soils may not be totally destroyed. For sanitizers to be effective when combined with cleaning compounds, the temperature of the cleaning solution should be 55°C or lower, and the soil should be light.

Desired Sanitizer Properties:

The ideal sanitizer should have the following properties:

- Microbial destruction properties of uniform, broad-spectrum activity against vegetative bacteria, yeasts, and molds to produce rapid kill
- Environmental resistance (effective in the presence of organic matter [soil load], detergent and soap residues, and water hardness and pH variability)
- Good cleaning properties
- Nontoxic and nonirritating properties
- Water solubility in all proportions
- Acceptability of odor or no odor
- Stability in concentrated and use dilution
- Ease of use
- Ready availability
- Inexpensive
- Ease of measurement in use solution

A standard chemical sanitizer cannot be effectively utilized for all sanitizing requirements. The chemical selected as a sanitizer should pass the Chambers test (also referred to as the *sanitizer efficiency test*): Sanitizers should produce 99.999% kill of 75 million to 125 million *Escherichia coli* and *Staphylococcus aureus* within 30 seconds after application at 20°C. The pH at which the compound is applied can influence the effectiveness of the sanitizer.

Chemical sanitizers are normally divided according to the agent that kills the microorganisms.

Chlorine Compounds

Liquid chlorine, hypochlorites, inorganic and organic chloramines, and chlorine dioxide function as sanitizers. Their antimicrobial activity varies.

1. Chlorine:

Chlorine gas may be injected slowly into water to form the antimicrobial form, hypochlorous acid (HOCl). Liquid chlorine is a solution of sodium hypochlorite (NaOCl) in water. Hypochlorous acid is 80 times more effective as a sanitizing agent than an equivalent concentration of the hypochlorite ion.

Chlorine is more stable at a high pH, but is less effective. Hypochlorous acid, the most active of the chlorine compounds, kills the microbial cell through inhibiting glucose oxidation by chlorine-oxidizing sulfhydryl groups of certain enzymes important in carbohydrate metabolism. Aldolase was considered to be the main site of action, owing to its essential nature in metabolism.

Other modes of chlorine action that have been proposed are:

- (1) Disruption of protein synthesis
- (2) Oxidative decarboxylation of amino acids to nitrites and aldehydes
- (3) Reactions with nucleic acids, purines, and pyrimidines
- (4) Unbalanced metabolism after the destruction of key enzymes
- (5) Induction of deoxyribonucleic acid (DNA) lesions with the accompanying loss of DNA-transforming ability
- (6) Inhibition of oxygen uptake and oxidative phosphorylation, coupled with leakage of some macromolecules
- (7) Formation of toxic N-chlor derivatives of cytosine
- (8) Creation of chromosomal aberrations.



Chlorinated isocyanurate is a highly stable, rapidly dissolving chlorine carrier that releases one of its two chloride ions to form NaOCl in aqueous solution.

Chlorine is known to be effective as a sanitizer for mechanically polished stainless steel, unabraded electropolished stainless steel, and the polycarbonate surfaces, reducing self-populations to less than 1.0 log CFU/cm².

2. Hypochlorites:

Calcium hypochlorite and sodium hypochlorite are the major compounds of the hypochlorites. These sanitizers are effective in deactivating microbial cells in aqueous suspensions.

Bacterial spores are more resistant than vegetative cells to hypochlorites. 200 ppm is effective for numerous surfaces; 800 ppm is suggested for porous areas.

Calcium hypochlorite, sodium hypochlorite, and brands of chlorinated trisodium phosphate may be applied as sanitizers after cleaning. Organic chlorine-releasing agents are sodium dichloroisocyanurate and dichlorodimethylhydantoin, can be formulated with cleaning compounds. These compounds appear to act through protein denaturation and enzyme inactivation.

Chlorine sanitizers are effective against gram-positive and gram-negative bacteria, and conditionally against certain viruses and spores. They are low temperature tolerant.

3. Chloramines

Inorganic chloramines are compounds formed from the reaction of chlorine with ammonia nitrogen.

Organic chloramines are formed through the reaction of hypochlorous acid with amines, imines, and imides.

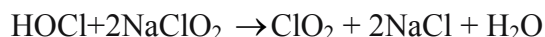
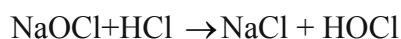
Bacterial spores and vegetative cells are more resistant to chloramine than to the hypochlorites.

Chloramine T apparently releases chlorine slowly. As a result, its lethal effects are slow when compared with the hypochlorites. Other chloramine compounds are as effective as, or more effective than, the hypochlorites in deactivating microorganisms.

4. Chlorine dioxide (ClO₂):

Chlorine dioxide is known to have 2.5 times the oxidizing power of chlorine. This compound is not as effective as chlorine at pH 6.5, but at pH 8.5, ClO₂ is the most effective. Thus, ClO₂ appears to be less affected by alkaline conditions and organic matter than hypochlorites, making it a viable agent for sewage treatment. Examples of how chlorine dioxide sanitizers are produced are indicated by the reactions that follow.

Chlorine dioxide is effective against a broad spectrum of microorganisms, including bacteria, viruses, and spore formers.



It is active over the broad pH range normally encountered in food facilities and more tolerant of organic matter than chlorine. This compound is less corrosive than other chlorine sanitizers because of the low concentration necessary to be effective and produces less “undesirable” chlorinated organics.

The major disadvantages of chlorine dioxide include cost, difficulty of handling, sensitivity to light and temperature, and potential safety and toxicity limitations.

The U.S. Food and Drug Administration (FDA) have approved the use of stabilized chlorine dioxide for sanitizing of food processing equipment. Industrial applications include a no-rinse sanitizer at 100 ppm, poultry chill tanks at 3 to 5 ppm, and drinking water treatment.

Oxine has gained recent interest as a sanitizer. It differs from generated ClO_2 as it is formulated from scratch, using a proprietary process, as opposed to being converted from chlorite. Increased microbial kill is possible by adjusting the ratio of chlorite and chlorine dioxide, and of other oxychlorine species, through the formation of oxine.

Chlorine compounds have the following advantages over other sanitizers:

1. They are effective against a variety of bacteria, fungi, and viruses.
2. They include fast-acting compounds that will pass the Chambers test at a concentration of 50 ppm in the required 30 seconds.
3. They are the cheapest sanitizers (if inexpensive chlorine compounds are used).
4. They are available in liquid or granular form.
5. They are unaffected by hard-water salts (except when slight variations, due to pH, exist).
6. High levels of chlorine may soften gaskets and remove carbon from rubber parts of equipment.
7. Toxic by-products are not produced.
8. They are less corrosive than chlorine.

Disadvantages:

1. They are unstable and drive off rather rapidly with heat or contamination with organic matter.
2. Their effectiveness decreases with increased solution pH.
3. They are corrosive to stainless steel and other metals.
4. They must be in contact with food-handling equipment, especially on any type of dishes, for only a short time to prevent corrosion.

5. They deteriorate during storage when exposed to light or to a temperature above 60°C.
6. Solutions at a lower pH can form toxic and corrosive chlorine gas (Cl₂).
7. Concentrated in the liquid form, they may be explosive.
8. Chlorine is irritating to the skin and mucous membranes.
9. The environmental impact is questionable because of the formation of potentially toxic organochlorine by-products.

Chlorine reacts with naturally occurring organic materials primarily humic acids and water which result in the formation of suspected carcinogenic trihalomethane compounds

Iodine Compounds:

The diatomic iodine is the major active antimicrobial agent, which disrupts bonds that hold cell proteins together and inhibits protein synthesis. Generally, free elemental iodine and hypoiodous acid are the active agents in microbial destruction.

The major iodine compounds used for sanitizing are iodophors, alcohol-iodine solutions, and aqueous iodine solutions. The two solutions are normally used as skin disinfectants.

The **iodophors** have value for cleaning and disinfecting equipment and surfaces, and as a skin antiseptic. Iodophors are also used in water treatment.

The iodophor complex releases an intermediate triciodide ion, which, in the presence of acid is rapidly converted to hypoiodous acid and diatomic iodine.

Both the hypoiodous acid and diatomic iodine are the active antimicrobial forms of an iodophor sanitizer. Ionic surface-active agents (surfactants) are compounds composed of two principal functional groups—a lipophilic portion and a hydrophilic portion. When placed in water, these molecules ionize, and the two groups induce a net charge to the molecule, which results in either a positive or a negative charge for the surfactant molecule. Cationic and anionic sanitizers have similar modes of action.

When elemental iodine is complexed with nonionic surface-active agents such as nonyl phenoethylene oxide condensates or a carrier such as polyvinylpyrrolidone, the watersoluble complexes known as *iodophors* are formed. Iodophors, the most popular forms of iodine compounds used today, have greater bactericidal activity under acidic conditions.

Bromine Compounds

Bromine has been used alone or in combination with other compounds, more in water treatment than as a sanitizer for processing equipment and utensils. At a slightly acidic to normal pH, organic chloramine compounds are more effective in destroying spores (such as *B. cereus*) than

are organic bromine compounds, but chloramine with bromine tends to be less affected by an alkaline pH of 7.5 or higher.

Quaternary Ammonium Compounds

The quaternary ammonium compounds, frequently called the *quats* are used most frequently on floors, walls, furnishings, and equipment. They are good penetrants and, thus, have value for porous surfaces. They are natural wetting agents with built-in detergent properties and are referred to as *synthetic surface-active agents*. Thus, they can be applied through foaming. The most common agents are the cationic detergents, which are poor detergents but excellent germicides.

Quaternary ammonium compounds are very effective sanitizers for the destruction of *Listeria monocytogenes* and effective in reducing mold growth. They are stable with a long shelf life. The quats are ammonium compounds in which four organic groups are linked to a nitrogen atom that produces a positively charged ion (cation). The mechanism of germicidal action is surface-active nature of the quat surrounds and covers the cell's outer membrane, causing a failure of the wall, which consequently causes leakage of the internal organs and enzyme inhibition.

The quats act against microorganisms differently than do chlorine and iodine compounds. They form a residual antimicrobial film after being applied to surfaces.

The quats do not kill bacterial spores but can inhibit their growth. Quaternary ammonium compounds are more stable in the presence of organic matter than are chlorine and iodine sanitizers.

The quaternary ammonium compounds include alkyldimethylbenzylammonium chloride and alkyldimethylethylbenzylammonium chloride

The quats have the following major advantages

- Colorless, odorless and nontoxic
- Stable against reaction with organic matter
- Resistant to corrosion of metals and not affected by hard water
- Stable against temperature fluctuation with a long shelf life
- Nonirritating to the skin
- Effective at a high pH with detergency and soil penetration ability
- Effective against mold growth
- Good surfactants that provide a residual antimicrobial film

They have these disadvantages:

- Limited effectiveness (including ineffectiveness against most gram-negative microorganisms except *Salmonella* and *E. coli*) with low hard-water tolerance and low-temperature activity

- Less effectiveness against bacteriophage
- Incompatibility with soaps and anionic type synthetic detergents since they are cationic molecules
- Film forming on food-handling and food-processing equipment
- Excessive foaming in mechanical applications and not recommended for use as cleaning-in-place sanitizers

Acid Sanitizers

Acid sanitizers, which are considered to be toxicologically safe and biologically active, are frequently used to combine the rinsing and sanitizing steps.

Organic acids, such as acetic, peroxyacetic, lactic, propionic, and formic acid, are most frequently used. Peroxyacetic acid compounds like acetic acid, octanoic acid and water are used at such low concentrations that there is no residual vinegar flavor. The acid neutralizes excess alkalinity that remains from the cleaning compound, prevents formation of alkaline deposits, and sanitizes.

Because bacteria have a positive surface charge, and negatively charged surfactants react with positively charged bacteria, their cell walls are penetrated, and cellular function is disrupted. These compounds are especially effective on stainless steel surfaces or where contact time may be extended and have a high antimicrobial activity against psychrotrophic microorganisms.

a. Peroxy Acid Sanitizers

The peroxy acid-hydrogen peroxide sanitizers represent a newer class of sanitizers.. Peroxy acid is a strong, fast- acting sanitizer that works on the same basis as chlorine-based sanitizers, through oxidation. It is EPA registered as a no-rinse food-contact surface sanitizer at the use dilution specified on the label. This sanitizer appears to be one of the most effective of those compounds available for protection against biofilms.

They leave no residues and are generally noncorrosive to stainless steel and aluminum in normal surface applications

Mixed Peroxy Acid/Organic Acid Sanitizers

The mixed peroxy acid/organic acid compounds are the next generation of peroxy acid-based sanitizers. This composition is based on the synergistic combination of organic acids and the original peroxyacetic acid. Generally, these products have the same advantages and disadvantages as the basic peroxy acid compounds. The mixed peroxy acid/organic acid sanitizers are generally more effective against various yeasts and molds than the basic peroxy acids.

Acid Anionic Sanitizers

These sanitizers are formulated with:

- Anionic surfactants (negatively charged)
- Acids phosphoric acid organic acids

Acid anionic sanitizers act rapidly and kill a broad spectrum of bacteria and have good bacteriophage activity. They have good stability, minimal odor, are nonstaining, effective in a wide temperature range, and are not affected by water hardness. Because this sanitizer is effective against yeasts—such as *Candida*, *Saccharomyces*, and *Hansenula*, and molds—such as *Penicillium*, *Aspergillus*, *Mucor*, and *Geotrichum*, it has gained acceptance in the soft drink and brewing industry. Disadvantages are high cost, odor, irritancy, tendency to corrode iron and other metals, and lower effectiveness against yeasts and molds than some sanitizers.

Acid-Quat Sanitizers:

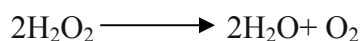
Organic acid sanitizers formulated with quaternary ammonium compounds were marketed as acid-quat sanitizers. This sanitizer is effective—especially against *L. monocytogenes*. A limitation of this type of sanitizer is that it is expensive when compared with the halogens.

LECTURE-24

Sanitation-Ozone, hydrogen peroxide, Activated Lactoferrin and Acidified sodium chlorite-Factors influencing efficacy of sanitizers.

Hydrogen Peroxide:

A hydrogen peroxide-based powder in 3% and 6% solutions is effective against biofilms. This antibacterial agent may be used on all types of surfaces, equipment, floors and drains, walls, steel mesh gloves, belts, and other areas where contamination exists. This compound is a weak acid that is formed to some extent by all aerobic organisms, and it is enzymatically degraded by the enzyme catalase.



It is a strong oxidising agent. It is used as a sterilant for food contact surfaces of olefin polymers and polyethylene in aseptic packaging systems. Also used at minimum levels as 0.08 or 0.05% in the flow injection pasteurization of egg white.

Ozone

Ozone, a molecule comprised of three oxygen atoms, is naturally occurring in the earth's upper atmosphere. It acts as a powerful and nonselective oxidant and disinfectant, (which indicates that it will attack any organic material that it contacts) and may control microbial and chemical hazards. Common by-products of ozonation are molecular oxygen, acids, aldehydes, and ketones.

This sanitizer does not cause a harmful residue or contaminated flavor. This sanitizer is a more powerful disinfectant than chlorine. It has been used safely and effectively in water treatment and is approved in the United States as Generally Regarded as Safe (GRAS) for treatment of bottled water and has been applied in the food industry in Europe during the past.

Ozone is being evaluated as a chlorine substitute. Because it oxidizes rapidly, it poses less environmental impact than some compounds.

Ozone is expensive, unstable, corrosive, temperature sensitive, very reactive, and should be generated as needed at the site of application. Ozone is most effective at a pH range of 6.0 to 8.5.

It is more effective in killing *Cryptosporidium parvum* than chlorine. The odor of ozone can be detected at 0.01ppm. The threshold limit for long term human exposure is 0.1ppm/day/work week (set as per the U.S. office for safety and Health Administration, OSHA).

The cell target for ozone is cell membrane. Ozone is GRAS for bottled water use, for use on a variety of fresh foods. Because of its strong oxidising agent capacity, it is not recommended for

red meats. A typical concentration of 0.1-0.5ppm is effective against gm+ve bacteria, gram -ve bacteria, protozoa, and viruses.

Glutaraldehyde

This sanitizer has been used to control the growth of common gram-negative and gram-positive bacteria, as well as species of yeasts and filamentous fungi found in conveyor lubricants used in the food industry.

Activated lactoferrin: (ALF, Activin).

Lactoferrin is a normal component of fresh milk. Lactoferrin contains antimicrobial properties. Activin is more potent antimicrobial than plain lactoferrin. In the activated form, lactoferrin is immobilized on food grade polysaccharides such as carrageenan and solubilized in a citric/bicarbonate buffer with NaCl.

Its antimicrobial activity is due to its capacity to chelate Fe^{2+} along with HCO_3^- . It binds to cell surfaces and has high affinity for the outer membrane proteins of Gm-ve bacteria. It also inhibits growth and neutralizes endotoxins. It is also active against DNA and RNA viruses.

Acidified Sodium Chlorite:

Acidified Sodium Chloride (ASC) is a product of citric or phosphoric acid and NaCl. It is used as spray or dip at concentrations of 1,000 to 1,000 ppm. The antimicrobial species is a product of the dissociation of chlorite that breaks or disrupts oxidative bonds on the surface of cell membranes in nonspecific manner. It has been approved by U.S Food and drug administration as a sanitizer for poultry, redmeat surfaces, seafoods, some fruits and vegetables and some processed meats. It may be used in water or ice. Acidic calcium sulfate is a closely related preparation.

Microbicides

The microbicide, 2-methyl-5-chloro-2-methyl isothiazolone, has potential for the control of *L. monocytogenes* on product conveyors. This microbicide has been found to be effective against *L. monocytogenes* (10-ppm). This biocide kills microorganisms quickly at a pH higher than 9.0.

Sanitizer Application

1. Chlorine --- All food-contact surfaces, spray, CIP, fogging
2. Iodine-----All food-contact surfaces, approach as a hand dip
3. Peracetic acid ---- All food-contact surfaces, CIP, especially cold temperature and carbon dioxide environments
4. Acid anionics ----All food-contact surfaces, spray, combines sanitizing and acid rinse into one operation

5. Quaternary ----- All food-contact surfaces, mostly used for environmental control; walls, ammonium drains, tiles compounds

Physical–chemical factors influencing efficacy of Sanitizers

The efficacy of sanitizers (especially chemical sanitizers) is affected by physical–chemical factors such as:

Exposure time: The death of a microbial population follows a logarithmic pattern, indicating that if 90% of a population is killed in a unit of time, the next 90% of the remaining is destroyed in the next unit of time, leaving only 1% of the original number. Microbial load and the population of cells having varied susceptibility to the sanitizer due to age, spore formation, and other physiological factors determine the time required for the sanitizer to be effective.

Temperature: The growth rate of the microorganisms and the death rate due to chemical application will increase as temperature elevates. A higher temperature generally lowers surface tension, increases pH, decreases viscosity, and creates other changes that may help bactericidal action. An exception is the iodophors that vaporize above 50°C. These chemicals are more aggressive to surfaces, especially elastomers and gasketing materials, as the temperature rises. Thus, chemical sanitizers should be applied at ambient temperatures, ideally 21 to 38°C

Concentration: Increased sanitizer concentration enhances the rate of destruction of the microorganisms.

pH: The activity of antimicrobial agents occurring as different species within a pH range may be dramatically influenced by relatively small changes in the pH of the medium. Chlorine and iodine compounds generally decrease in effectiveness with an increase in pH.

Equipment cleanliness: Hypochlorites, other chlorine compounds, iodine compounds, and other sanitizers can react with the organic materials of soil that have not been removed from equipment and other surfaces. Failure to clean surfaces properly can reduce the effectiveness of a sanitizer. Oxidizing chemicals react with organic materials, such as soils, reducing their effectiveness against target microorganisms.

Water hardness:

A sanitizer is affected by water composition, which can make the sanitizer chemically inactive or buffer the pH and diminish effectiveness. Quaternary ammonium compounds are incompatible with calcium and magnesium salts and should not be used with over 200 parts per million (ppm) of calcium in water or without a sequestering or chelating agent. As water hardness increases, the effectiveness of these sanitizers decreases.

Microbial population:

All sanitizers are not equally effective against all microorganisms. Cells in the spore state or in a biofilm are more resistant than those in the vegetative and freely suspended state. Beverage plants with yeasts and molds as their primary contaminants may need a different sanitizer than fluid milk plants, which are primarily concerned with psychrotrophic spoilage bacteria. Since a sanitizer can only reduce the number of bacteria, the higher the initial number present, the higher the amount of possible survivors. High numbers can overwhelm the sanitizer.

Bacterial attachment:

The attachment of certain bacteria to a solid surface provides an increased resistance to chlorine. Other factors, such as nutrient limitation (stringent response), also do so, and with attachment, the resultant resistance to chlorine is increased.

LECTURE-25

Food safety-Indicators of food microbial Quality and safety-Coliforms, Enterococci, Bifidobacteria, Coliphages/Enteroviruses, predictive Microbiology/ Microbial modeling.

Microbial Standards of Processed and preserved Foods

The Center for disease control (CDC) investigates each documented out break of food borne disease and attempts to determine not only the specific microorganisms and foods involved but also the events which led to the out break.

Indicators of Food Safety:

Microbial indicators are employed more often to assess food safety and sanitation than quality. Ideally a food safety indicator should meet certain important criteria.

1. It should be easily and rapidly detectable
2. It should be easily distinguishable from other members of the food biota.
3. It should have a history of constant association with the pathogen whose presence it is to indicate
4. It should always be present when the pathogen of concern is present
5. It should be an organism whose numbers ideally should correlate with those of the pathogen of concern.
6. It should possess growth requirements and a growth rate equaling or exceeding that of the pathogen
7. It should have a die-off rate at least it parallels that of the pathogen and ideally persists slightly longer than pathogen of concern.
8. It should be absent from foods that are free of the pathogen except perhaps at certain minimum numbers.

Coli forms:

- Etiologic agent of cholera in 1885.
- Originally named as bacterium coli concerne because it was present in the stools of each patient he examined.
- Schardinger – suggested the use of this organism as an index of fecal pollution. Rapidly than individual water borne pathogens.
- A test for this organism as a measure of drinking water portability was suggested in 1895 by T. Smith.

- They produce metallic sheen on colonies of enterobacter, citrobacter, Enterobacter, klebsiella, Raoultella.
- IMVIC formula is the classic method used.

I = Indole Production

M = Methyl red reaction

V = Voges – Proskauer reaction

C = Citrate utilization

Coliform criteria and standards:

1. Grade A pasteurized milk & milk products including cultured products not over 10/ml
2. Certified raw milk not more 10/ml. Certified pasteurized milk not over 1/ml
3. Pre cooked & partially cooked frozen foods not over 10/ml
4. Crab meat not over 100/ml
5. Custard filled items not over 100/ml

IEMSF – International commission on the microbiological specifications for foods.

Enterococci:

E. Faecalis (M. ovalis)

- Pollution indicators for water:
 1. They generally do not multiply in water especially if the organic matter content is low.
 2. They are generally less numerous in human feces than E.coli
 3. The enterococci die off at a slower rate than coliforms in waters.
- E. Faecium present in hogs & wild boars, E. Faecalis present in feces of variety of mammals
- Grows at 10°C - 45°C
- pH – 9.6
- Mole % GTC – 37 – 45
- Enterococci are more fastidious in their nutritional requirements for more growth factors
- Although they are aerobes but they do not produce catalase.

Distribution:

E. faecalis & E. faecium – present in fecal matters.

E. hirae and E. durans – found more often in poultry and cattle.

E. gallinarum – found only in poultry

E. cecorum – isolated from chicken cecae

E. columbae – pigeon intestines

E. saccharolyticum – from cows

E. avium – mammalian and chicken feces.

E. casseliflavus – silage soils and plants.

E. mundtii – on cows, hands of milkers, soils and plants

E. hirae – chicken and pig intestines

E. dispar – certain human specimens.

Relationship sanitary quality of foods:

Enterococci are better indicators of food sanitary quality especially for frozen foods compared to coliforms.

Bifidobacteria:

Tissier (1908) identified and named as *Bacillus bifidus*. Later it was named as *Lactobacillus bifidus* and contently known as *Bifidobacterium bifidum*.

- Commonly present in stools
- They are gram positive anaerobic bacteria as indicators of fecal pollution, especially of waters.
- Some bifido bacteria are employed in the production of fermented milks, yogurt and other food products and they are believed to provide some health benefits.
- Growth conditions – Temperature min 25-28°C and max 43-45°C and P^H 5-8
- Produce lactic and acetic acids as the major end products of their carbohydrate metabolism.

Distribution:

- Found in human feces at higher lutes per gram ($10^8 - 10^9$) than *E. coli* ($10^6 - 10^7$) and this makes them more attractive as indicators of human fecal pollution.
- By using the bifido bacteria, it is possible to determine their origin among the following three sources: human feces, animal feces or environmental conditions.
- Gavini et al (1991) devised the method to distinguish between human and animal strains and it divides bifido bacteria into seven groups.
- Human origin belong to I, III and VII.
- *B. adolescentis* and *B. longum* are found often isolated in highest numbers.

Coliphages / Enteroviruses:

- Indirect indicators.

- A coli phage assay procedure for water samples that contain five or more phases / 100ml and that can be completed in 4-6 hours is described in “Standard methods for the examination of water and waste water”.

Predictive microbiology or Microbial modeling:

- Microbial modeling or predictive microbiology is a rapidly emerging sub discipline that entails the use of mathematical models/equations to predict the growth and/or activity of microorganisms in a food product over time.
- It is applied in heat process calculations in the canning of low acid foods, food poisoning and food spoilage organisms by the use of more sophisticated mathematical / computer models that can handle more growth parameters.
- The effective applications of predictive microbiology require the selection of appropriate to reflect the effect of growth parameters. Among the many models that have been proposed and tested are two kinetic models – the non linear arrhenius and Belebradek types.
- Non linear Arrhenius model is applied with the dependent variable expressed as in (logarithm) rate latter square root model; the dependent variable is expressed as $\sqrt{\quad}$ rate.
- Computer software packages for predictive microbiology are available from private and commercial sources.
- One of the simplest applications of predictive microbiology is the use of Monte Carlo simulation. Based on collected dates to predict shelf life/safety relative to changes in environmental parameters such as P^H , a_w etc.

LECTURE-26

Risk assessment and management during food preparation- HACCP –prerequisite programmes, definitions, HACCP principles, Flow diagrams, Application of HACCP principles, Limitations of HACCP

Although it may not be possible to achieve a zero tolerance for all such organisms under good manufacturing practices (GMP), the production of foods with the lowest possible numbers is the desirable goal.

HACCP (Hazard Analysis Critical Control Point) System.

HACCP is a system that should lead to the production of microbiologically safe foods by analyzing for the hazards of raw materials-those that may appear throughout processing and those that may occur from consumer abuse.

It is a proactive, systematic approach to controlling food borne hazards. Although some classic approaches to food safety rely heavily on end product testing, the HACCP system places emphasis on the quality of all ingredients and all process steps on the premise that safe products will result if these are properly controlled. The system is thus designed to control organisms at the point of production and preparation.

Prerequisite Programmes:

Prerequisite programs include a wide range of activities and events that may have an impact on HACCP system for a specific food product even though they are not parts of the HACCP system.

Prerequisite programs include concerns and aspects of the entire food environment before the HACCP system is initiated. They include the suitability of facilities, control of suppliers, safety and maintenance of production equipment, cleaning and sanitation of equipment and facilities, personal hygiene of employees, control of chemicals, pest control etc., These prerequisites include good manufacturing practices (GMP) and they should be brought up to acceptable standards before the HACCP system is initiated.

Definitions:

The following terms and concepts are valuable in the development and execution of HACCP system and are taken from International Commission on Microbiological Specifications for Foods (ICMSF) and/or and/or National Advisory Committee on the Microbiological Criteria for Foods (NACMCF).

Control point: Any point in a specific food system where loss of control does not lead to an unacceptable health risk.

Critical control point (CCP): Any point or procedure in a food system where control can be exercised and a hazard can be minimized or prevented.

Critical limit: One or more prescribed tolerances that must be met to ensure that a CCP effectively controls a microbiological health hazard.

CCP decision tree: A sequence of questions to assist in determining whether a control point is a CCP.

Corrective action: Procedures followed when a deviation occurs.

Deviation: Failure to meet a required critical limit for a CCP.

HACCP plan: The written document that delineates the formal procedures to be followed in accordance with these general principles.

Hazard: Any biological, chemical, or physical property that may cause an unacceptable consumer health risk (unacceptable contamination, toxin levels, growth, and/or survival of undesirable organisms).

Monitoring: A planned sequence of observations or measurements of critical limits designed to produce an accurate record and intended to ensure that the critical limit maintains product safety.

Risk category: One of six categories prioritizing risk based on food hazards.

Validation: That element of verification focused on collecting and evaluating scientific and technical information to determine whether the HACCP plan, when properly implemented, will effectively control the hazards.

Verification: Methods, procedures, and tests used to determine whether the HACCP system is in compliance with the HACCP plan.

HACCP principles:

Although interpreted variously, the ICMSF and NACMCF view HACCP as a natural and systematic approach to food safety and as consisting of the following seven principles:

1. Assess the hazards and risks associated with the growing, harvesting, raw materials, ingredients, processing, manufacturing, distribution, marketing, preparation, and consumption of the food in question.
2. Determine the CCP(s) required controlling the identified hazards.
3. Establish the critical limits that must be met at each identified CCP.
4. Establish procedures to monitor the CCP(s).

5. Establish corrective actions to be taken when there is a deviation identified by monitoring a given CCP.

6. Establish procedures for verification that the HACCP system is working correctly.

7. Establish effective record-keeping systems that document the HACCP plan.

Each of these principles is discussed in more detail below:

Principle 1: Assess Hazards and Risks

Hazards and risks may be assessed for individual food ingredients from a flow diagram or by ranking the finished food product by assigning to it a hazard rating from A through F.

A plus sign (+) is assigned when a hazard exists. Six hazard categories have been defined, representing an expansion of the three proposed by the National Research Council (NRC) for salmonellae control. It is presented here for historical purposes:

A. This is a special class of foods that consist of non sterile products designated and intended for consumption by individuals at risk, including infants, the aged, infirm, and immunoincompetent.

B. The product contains "sensitive" ingredients relative to microbiological hazards (e.g., milk, fresh meats).

C. There is no controlled processing step (such as heat pasteurization) that effectively destroys harmful microorganisms.

D. The product is subject to recontamination after processing but before packaging (e.g., pasteurized in bulk and then packaged separately).

E. Substantial potential for abusive handling exists in distribution and/or by consumers that could render the product harmful when consumed (e.g., products to be refrigerated are held above refrigerator temperatures).

F. There is no terminal heat process after packaging or when cooked in the home.

Next, the formulated product should be assigned to one of six hazard categories, expanded from four suggested by the NRC.

VI. A special category that applies to non sterile products designated and intended for individuals in hazard category A.

V. Food products subject to all five general hazard characteristics. (B, C, D, E, and F)

IV. Food products subject to any four general hazard characteristics.

III. Products subject to any three of the general hazard characteristics.

II. Products subject to any two general hazard characteristics.

I. Products subject to anyone of the general hazard characteristics.

0. Products subject to no hazards

Principle 2: Determine CCP(s) (CCP: Critical control point)

The ICMSF recognized two types of CCPs:

CCPI: To ensure control of a hazard.

CCP2: To minimize a hazard.

Typical of CCPs are the following:

1. Heat process steps where time-temperature relations must be maintained to destroy given pathogens.
2. Freezing and time to freezing before pathogens can multiply.
3. The maintenance of pH of a food product at a level that prevents growth of pathogens.
4. Employee hygiene.

Principle 3: Establish Critical Limits

A critical limit is one or more prescribed tolerances that must be met to ensure that a CCP effectively controls a microbiological hazard.

This could mean keeping refrigeration temperatures within a certain specific and narrow range, or making sure that a certain minimum destructive temperature is achieved and maintained long enough to effect pathogen destruction.

Examples of the latter include adherence to the temperatures as following for the control of the respective organisms.

Cooking parameters	Temperatures
1. Cooked beef and roast beef (121 min at 130 ⁰ F to instantaneous at 145 ⁰ F)	130-145 ⁰ F (54.4-62.7 ⁰ C)
2. Baked meat loaf	160 ⁰ F (71.7 ⁰ C)
3. Baked pork cut	170 ⁰ F (76.7 ⁰ C)
4. Poultry products	160 ⁰ F (71.7 ⁰ C)
Cooling parameters	
1. Refrigerated storage temperature and internal temperature control point	40 ⁰ F (4.447 ⁰ C)
2. Recommended refrigerated storage temperature for periods exceeding 1 week.	35 ⁰ F (1.7 ⁰ C)

Principle 4: Establish Procedures to Monitor CCPs

The monitoring of a CCP involves the scheduled testing or observation of a CCP and its limits. Monitoring results must be documented. If, for example, the temperature for a certain process step should not exceed 40°C, a chart recorder may be installed.

Microbial analyses are not used to monitor since too much time is required to obtain results. Physical and chemical parameters such as time, pH, temperature, and water activity (aw) can be quickly determined and the results obtained immediately.

Principle 5: Establish Corrective Actions

Establish corrective actions to be taken when deviations occur in CCP monitoring. The actions taken must eliminate the hazard that was created by any deviation from the plan. If a product is involved that may be unsafe as a result of the deviation, it must be removed. Although the actions taken may vary widely, in general they must be shown to bring the CCP under control.

Principle 6: Establish Procedures for Verification

Establish procedures for verification that the HACCP system is working correctly. Verification consists of methods, procedures, and tests used to determine that the system is in compliance with the plan.

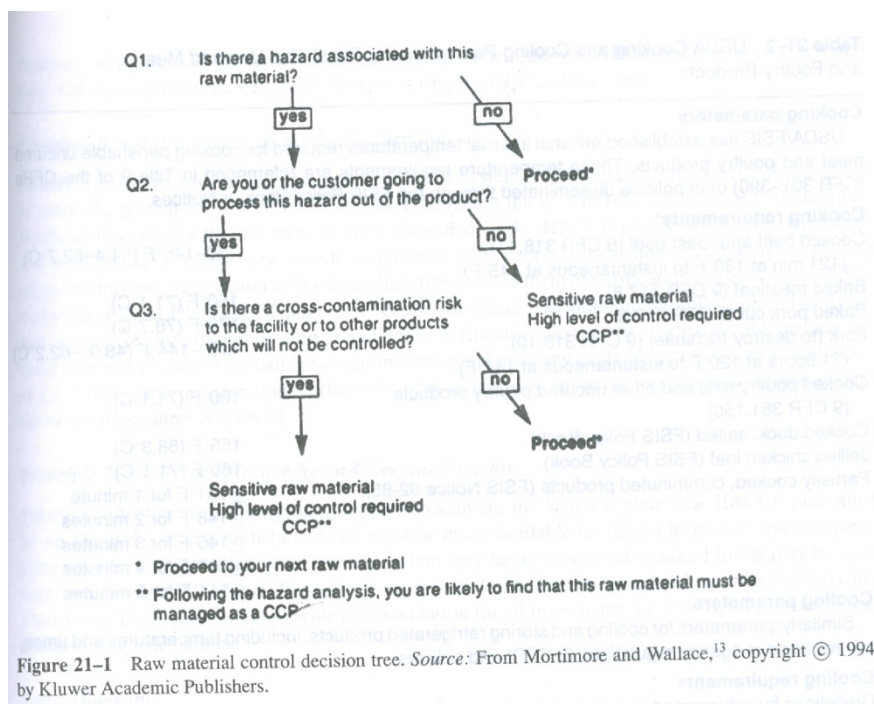
Verification confirms that all hazards were identified in the HACCP plan when it was developed, and verification measures may include compliance with a set of established microbiological criteria when established.

Verification activities include the establishment of verification inspection schedules, including review of the HACCP plan, CCP records, deviations, random sample collection and analysis, and written records of verification inspections.

Verification inspection reports should include the designation of persons responsible for administering and updating the HACCP plan, direct monitoring of CCP data while in operation, certification that monitoring equipment is properly calibrated, and deviation procedures employed.

Principle 7: Establish Effective Record-Keeping Systems

Establish effective record-keeping systems to document the HACCP plan. The HACCP plan must be on file at the food establishment and must be made available to official inspectors upon request. Forms for recording and documenting the system may be developed, or standard forms may be used with necessary modifications. Typically, these may be forms that are completed on a regular basis and filed away. The forms should provide documentation for all ingredients, processing steps, packaging, storage, and distribution.



Flow Diagrams:

The development of an HACCP plan for a food establishment begins with the construction of a flow diagram for the entire process. The diagram should begin with the acquisition of raw materials and include all steps through packaging and subsequent distribution. A flow diagram for the production of frozen, cooked beef patties is illustrated in Figure 21-2. To begin the HACCP process, the three questions in Figure 21-1 should be raised. When this is done, the answer to all three is yes, as outlined below:

Q 1. Is there a hazard? Raw ground beef patties are known to be vehicles for *Escherichia coli* 0157:H7, *Toxoplasma gondii*, and salmonellae.

Q2. Will the hazard be processed out? This will be achieved in step 5 (cooking).

Q3. Is there a risk of cross-contamination? This can occur in steps 7, 8, and 10.

Application of HACCP Principles:

It deals with an application of the seven HACCP principles to the manufacture of frozen, cooked beef patties as outlined in Figure 21-2, and the steps referred to are those on the flow diagram.

Principle I-Hazards and Risks

Raw meat is a sensitive ingredient and the cooked product is subject to recontamination after processing and during distribution.

Principle 2-CCPs

An important concern about step 1 is the overall condition of the beef carcasses or cuts. The comments below are based on the assumption that the beef has been produced and handled under GMP. Step 5 is the indisputable CCP 1, since it can eliminate the hazards. CCP2s may be assigned to steps 6 and 8, and possibly to step 7.

Principle 3-Critical Limits

Temperature is the critical parameter from steps 1 to 9, and it consists of proper refrigeration temperature in steps 1 to 4; proper cooking temperature in step 5; freezing in steps 6 to 8; and heating in step 9. The overall objective is to keep the fresh beef at or below 40°F (4.4°C) at all times, cook patties to 160°F (71.7°C), freeze to -20°F or 0°C, and store at the same temperature.

Principle 4-Monitoring HACCP

Use chart recorders for steps 2 to 4, use thermometers for steps 5 and 6, and temperature recorders for step 8

Principle 5-Corrective Actions

These refer to deviations from critical limits identified during monitoring of CCPs. Specific corrective actions to be taken should be clearly spelled out. For example, if the target temperature in step 5 is not reached, will the batch be discarded, reprocessed, or assigned to another use?

Principle 6-Verification

Overall, this is an assessment of how effective the HACCP system is performing. Typically, some microbial analyses are in order—for example, were all relevant pathogens destroyed in step 5? Have the products in retail stores been contaminated after being cooked?

Principle 7-Record-Keeping

This should be done by product lot number in such a way that records are available to verify the events in steps 2 to 4. Where room temperatures are involved, chart recorder tracings should be kept.

A flow diagram for the production of roast beef is presented in Figure 21-3. Cooking is the most important CCP for this product (CCP1), followed by chilling and prevention of recontamination after cooking. The cooking temperature should reach 145°F (62.3°C) or otherwise be sufficient to effect a 4-log cycle reduction of *Listeria monocytogenes*. This will not destroy *Clostridium perfringens*

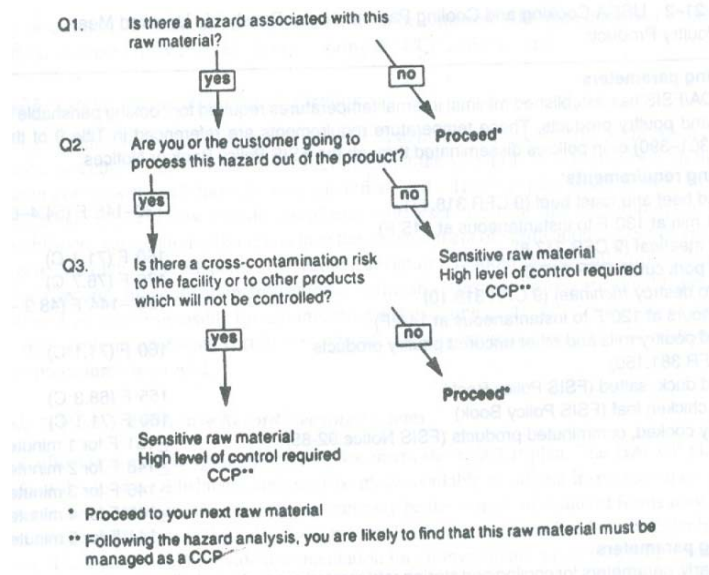


Figure 1: Raw material control decision tree

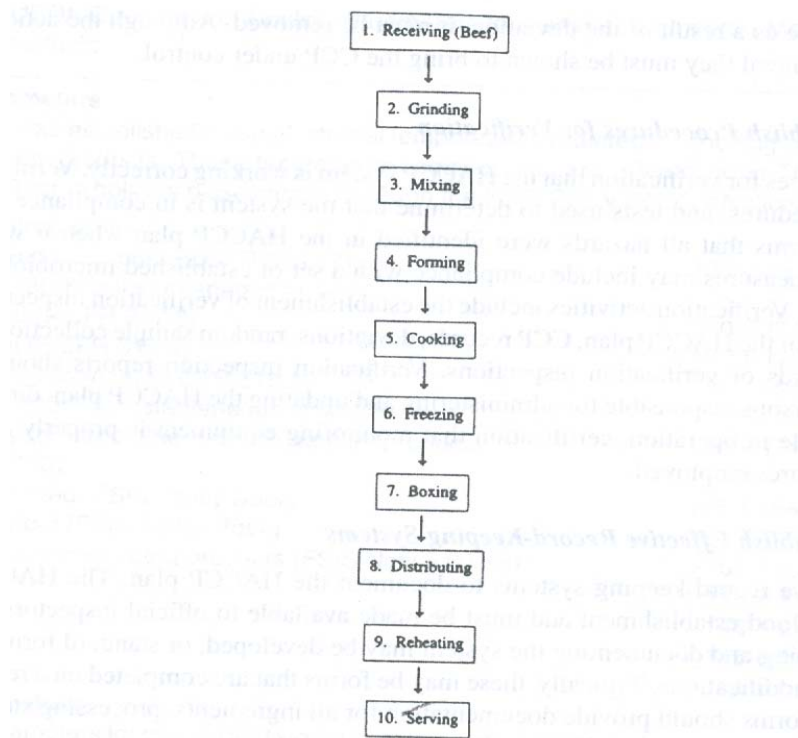


Figure 2: Example of a flow diagram for the production of production of frozen, cooked beef patties

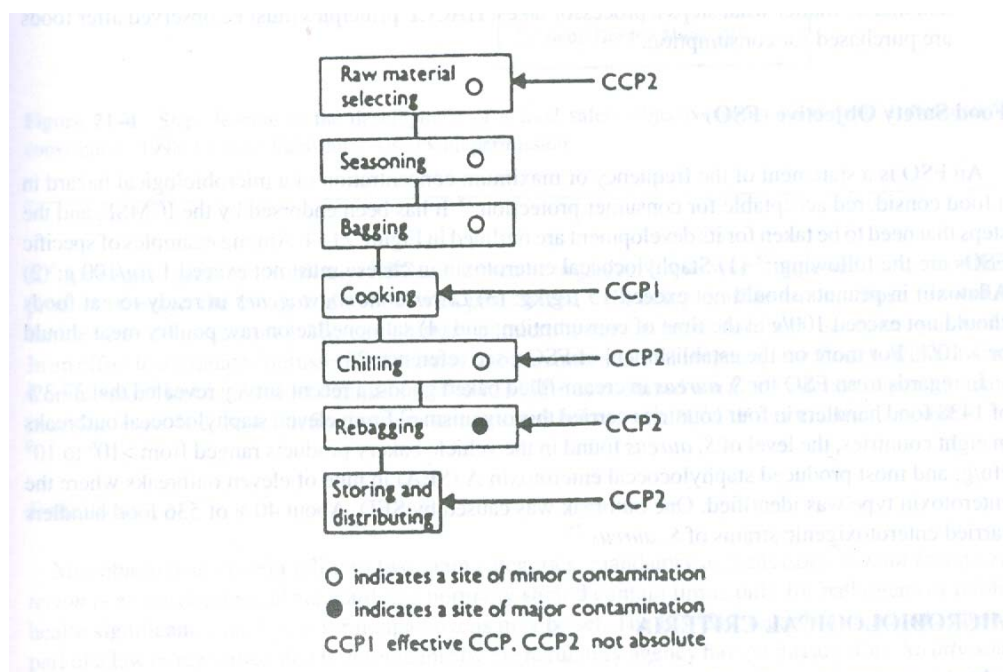


Figure 3: Flow diagram for the production of Roasted beef

Limitations of HACCP:

1. HACCP requires the education of nonprofessional food handlers, especially in the food service industry and in homes; whether this will be achieved remains to be seen. The failure of these individuals to get a proper understanding of HACCP could lead to its failure.
2. To be effective, this concept must be accepted not only by food processors but also by food inspectors and the public. Its ineffective application at any level can be detrimental to its overall success for a product.
3. It is anticipated that experts will differ as to whether a given step is a CCP and how best to monitor such steps. This has the potential of eroding the confidence of others in HACCP.
4. The adoption of HACCP by industry has the potential of giving false assurance to consumers that a product is safe, and, therefore, there is no need to exercise the usual precautions between the purchase and consumption of a product. Consumers need to be informed that most outbreaks of food borne illness are caused by errors in food-handling in homes and food service establishments and that no matter what steps a processor takes, HACCP principles must be observed after foods are purchased for consumption.

LECTURE-27

Risk assessment and management during food preparation- Food safety Objective (FSO), Microbiological criteria, definitions, sampling plans.

Food Safety Objective:

FSO is a statement of the frequency or maximum concentration of a microbiological hazard in a food considered acceptable for consumer protection.

It has been endorsed by the ICMSF, and the steps that need to be taken for its development are outlined in Figure 21-4.

Among examples of specific FSOs are the following:

- (1) Staphylococcal enterotoxin in cheese must not exceed 1 µg/100 g
- (2) Aflatoxin in peanuts should not exceed 15 µg/kg
- (3) *Listeria monocytogenes* in ready-to-eat foods should not exceed 100/g at the time of consumption
- (4) Salmonellae on raw poultry meat should be < 10%.

Microbiological Criteria

The concept that microbial limits be assigned to at least some foods to designate their safety or overall quality was suggested as early as 1903 by Marxer, who suggested an aerobic plate count (APC) limit of 10^6 for hamburger meat.

Similarly, APC and indicator organism limits were suggested for many other products through the 1920s and 1930s, with pasteurized milk being notable among those for which limits were widely accepted. In an effort to eliminate confusion and to agree upon an international language, the Codex Alimentarius Commission has established definitions.

Definitions:

Microbiological criteria fall into two main categories. and 2. Advisory

1. Mandatory: A mandatory criterion is a microbiological standard that normally should contain limits only for pathogens of public health significance, but limits for non pathogens may be set. The ICMSF regards a *standard* as being part of a law or regulation that is enforceable by the regulatory agency having jurisdiction.
2. Advisory: An advisory criterion is either a microbiological end product specification intended to increase assurance that hygienic significance has been met (it may include spoilage organisms), or a microbiological guideline that is applied in a food establishment at a point during or after processing to monitor hygiene (it, too may include non pathogens).

Before recommending a criterion, the ICMSF notes that each product must be in international trade, must have associated with it good epidemiological evidence that it has been implicated in foodborne disease, and have associated with it good evidence that a criterion will reduce the potential hazard(s) in Principle 2.

The Codex definition of a microbiological criterion consists of five components:

- (1) A statement of the organisms of concern and/or their toxins.
- (2) The analytical methods for their detection and quantitation
- (3) A sampling plan, including when and where samples are to be taken.
- (4) Microbiological limits considered appropriate to the food.
- (5) The number of sample units that should conform to these limits.

These five components are embodied in a sampling plan.

Sampling Plans:

A sampling plan is a statement of the criteria of acceptance applied to a lot based on appropriate examinations of a required number of sample units by specified methods. It consists of a sampling procedure and decision criteria and may be a two-class or a three-class plan.

Two class Plan: A two-class plan consists of the following specifications: n , c , m

Three Class Plan: A three-class plan requires n , c , m , and M ,

n = the number of sample units (packages, beef patties, and so forth) from a lot that must be examined to satisfy a given sampling plan.

c = the maximum acceptable number, or the maximum allowable number of sample units that may exceed the microbiological criterion m . When this number is exceeded, the lot is rejected.

m = the maximum number or level of relevant bacteria per gram; values above this level are either marginally acceptable or unacceptable. It is used to separate acceptable from unacceptable foods in a two-class plan, or, in a three-class plan, to separate good quality from marginally acceptable quality foods. The level of the organism in question that is acceptable and attainable in the food product is m . In the presence/absence situations for two-class plans, it is common to assign $m = 0$. For three-class plans, m is usually some nonzero value.

M = a quantity that is used to separate marginally acceptable quality from unacceptable quality foods. It is used only in three-class plans. Values at or above M in any sample are unacceptable relative to health hazard, sanitary indicators, or spoilage potential.

A two-class plan is the simpler of the two and in its simplest form may be used to accept or reject a larger batch (lot) of food in a presence/absence decision by a plan such as $n = 5$, $c = 0$,

where $n = 5$ means that five individual units of the lot will be examined microbiologically for, say, the presence of salmonellae, and $c = 0$ means that all five units must be free of the organisms by the method of examination in order for the lot to be acceptable. If any unit is positive for salmonellae, the entire lot is rejected. If it is desired that two of the five samples may contain coliforms, in a presence/absence test, for example, the sampling plan would be $n = 5$, $c = 2$. By this plan, if three or more of the five unit samples contained coliforms, the entire lot would be rejected. Although the presence/absence situations generally obtained for salmonellae, an allowable upper limit for indicator organisms such as coli forms is more often the case. If it is desired to allow up to 100 coliforms/g in two of the five units, the sampling plan would be $n = 5$, $c = 2$, $m = 10^2$. After the five units have been examined for coliforms, the lot is acceptable if no more than two of the five contain as many as 10^2 coliforms/g but is rejected if three or more of the five contain 10^2 coliforms/g. This particular sampling plan may be made more stringent by increasing n (e.g., $n = 10$, $c = 2$, $m = 10^2$) or by reducing c (e.g., $n = 5$, $c = 1$, $m = 10^2$). On the other hand, it can be made more lenient for a given size n by increasing c .

Whereas a two-class plan may be used to designate acceptable/unacceptable foods, a three-class plan is required to designate acceptable/marginally acceptable/unacceptable foods. To illustrate a typical three-class plan, assume that for a given food product, the standard plate count (SPC) shall not exceed 10^6 /g (M) or be higher than 10^5 /g from three or more of five units examined. The specifications are thus $n = 5$, $c = 2$, $m = 10^5$, $M = 10^6$. If any of the five units exceeds 10^6 /g, the entire lot is rejected (unacceptable). If not more than c sample units give results above m , the lot is acceptable. Unlike two-class plans, the three-class plan distinguishes values between m and M (marginally acceptable).

With either two- or three-class attribute plans, the number n and c may be employed to find the probability of acceptance (P_a) of lots of foods by reference to appropriate tables.

The decision to employ a two-class or three-class plan may be determined by whether presence/absence tests are desirable, in which case a two-class plan is required, or whether count or concentration tests are desired, in which case a three-class plan is preferred. The latter offers the advantages of being less affected by non random variations between sample units and of being able to measure the frequency of values in the m to M range.

LECTURE-28

Microbiological criteria for various food products- Sea foods, Milk products, Spices, Fruits and vegetables.

Microbiological Criteria for various food products:

Presented below are foods and food ingredients that are covered under microbiological standards of various organizations (in the United States) along with federal, state, and city standards in effect.

1. Standards for Starch and Sugar (National Canners Association)

A. Total thermophilic spore count: Of the five samples from a lot of sugar or starch none shall contain more than 150 spores per 10g, and the average for all samples shall not exceed 125 spores per 10g.

B. Flat-sour spores: Of the five samples, none shall contain more than 75 spores/10g, and the average for all samples shall not exceed 50 spores per 10g.

C. Thermophilic anaerobe spores: Not more than three (60%) of the five samples shall contain these spores, and in anyone sample, not more than four (65%) of the six tubes shall be positive.

D. Sulfide spoilage spores: Not more than two (40%) of the five samples shall contain these spores, and in anyone sample, there shall be no more than five colonies per 10 g (equivalent to two colonies in the six tubes).

2. Standard for "Bottlers" Granulated Sugar, Effective July 1, 1953 (American Bottlers of Carbonated Beverages)

A. Mesophilic bacteria: Not more than 200 per 10 g.

B. Yeasts: Not more than 10 per 10 g.

C. Molds: Not more than 10 per 10 g.

3. Standard for "Bottlers" Liquid Sugar, Effective in 1959 (American Bottlers of Carbonated Beverages). All figures based on dry-sugar equivalent (D.S.E.)

A. Mesophilic bacteria (a) Last 20 samples average IOU organisms or less per 10 g of D.S.E.; (b) 95% of last 20 counts show 200 or less per 10 g; (c) 1 of 20 samples may run over 200; other counts as in (a) or (b).

B. Yeasts: (a) Last 20 samples average 10 organisms or less per 10 g of D.S.E.; (b) 95% of last 20 counts show 18 or less per 10 g; (c) 1 of 20 samples may run over 18; other counts as in (a) and (b).

C. Molds: Standards like those for yeasts.

4. Standards for Dairy Products

A. From 1965 recommendations of the U.S. Public Health Service.

a. Grade A raw milk for pasteurization: Not to exceed 100,000 bacteria per milliliter prior to commingling with other producer milk; and not exceeding 300,000 per milliliter as commingled milk prior to pasteurization.

b. Grade A pasteurized milk and milk products (except cultured products): Not over 20,000 bacteria per milliliter, and not over 10 coliforms per milliliter.

c. Grade A pasteurized cultured products: Not over 10 coliforms per milliliter. Note: Enforcement procedures for (a), (b), and (c) require a three-out-of-five compliance by samples.

Whenever two of four successive samples do not meet the standard, a fifth sample is tested; and if this exceeds any standard, the permit from the health authority may be suspended. It may be reinstated after compliance by four successive samples has been demonstrated.

B. Certified milk (American Association of Medical Milk Commissions, Inc.)

a. Certified milk (raw): Bacterial plate count not exceeding 10,000 colonies per milliliter; coliform colony count not exceeding 10 per milliliter.

b. Certified milk (pasteurized): Bacterial plate count not exceeding 10,000 colonies per milliliter before pasteurization and 500 per milliliter in route samples. Milk not exceeding 10 coliforms per milliliter before pasteurization and 1 coliform per milliliter in route samples.

C. Milk for manufacturing and processing (USDA, 1955)

a. Class 1: Direct microscopic clump count (DMC) not over 200,000 per milliliter.

b. Class 2: DMC not over 3 million per milliliter.

c. Milk for Grade A dry milk products: must comply with requirements for Grade A raw milk for pasteurization (see above).

D. Dry milk

a. Grade A dry milk products: at no time a standard plate count over 30,000 per gram, or coliform count over 90 per gram (U.S. Public Health Service).

b. Standards of Agricultural Marketing Service (USDA):

(1) Instant nonfat: U.S. Extra Grade, a standard plate count not over 35,000 per gram, and coliform count not over 90 per gram.

(2) Nonfat (roller or spray): U.S. Extra Grade, a standard plate count not over 50,000 per gram; U.S. Standard Grade, not over 100,000 per gram

(3) Nonfat (roller or spray): Direct microscopic clump count not over 200 million per gram; and must meet the requirements of U.S. Standard Guide. U.S. Extra Grade, such as used for school lunches, has an upper limit of 75 million per gram.

c. Dried milk (International Dairy Federation proposed microbiological specifications, 1982).

Mesophilic count: $n = 5$, $c = 2$, $m = 5 \times \text{Hr}$, $M = 2 \times 10^5$ Coliforms: $n = 5$, $c = 1$, $m = 10$, $M = 100$

Salmonella: $n = 15$, $c = 0$, $m = 0$.

E. Frozen desserts

States and cities that have bacterial standards usually specify a maximal count of 50,000 to 100,000 per milliliter or gram. The U.S. Public Health Ordinance and Code sets the limit at 50,000 and recommends bacteriological standards for cream and milk used as ingredients. Few localities have coliform standards.

5. Standard for Tomato Juice and Tomato Products-Mold-count Tolerances (Food and Drug Administration)

The percentage of positive fields tolerated is 2% for tomato juice and 40% for other comminuted tomato products, such as catsup, puree, paste, and so forth. A microscopic field is considered positive when an aggregate length of not more than three mold filaments present exceeds one sixth of the diameter of the field (Howard mold count method). This method has also been applied to raw and frozen fruits of various kinds, especially berries.

Other criteria or Guideline:

1. Sampling plans and microbiological limits for nine products as recommended by ICMSF are presented in Table 21-3 (for an explanation of plan stringency or case,

The examples presented were selected to reflect different plan stringencies (for two- and three-class plans) and limits for a variety of organisms.

2. Suggested guidelines for further processed deboned poultry products studied in Canada.

3. Canadian criteria for cottage cheese and ice cream:

Coliforms: $n = 5$, $c = 1$, $m = 10$, $M = 10^3$ (for cottage cheese and ice cream) Aerobic plate count: $n = 5$, $c = 2$, $m = 10^5$, $M = 10^6$ (for ice cream only).

4. Recommended criteria for cooked ready-to-eat shrimp: 15

S. aureus: $n = 5$, $c = 2$, $m = 50$, $M = 50$; Coliforms: $n = 5$, $c = 2$, $m = 102$, $M = 10^3$

5. Recommended criteria for cooked ready-to-eat crabmeat:

S. aureus: $n = 5$, $c = 2$, $m = 10^2$, $M = 10$; Coliforms: $n = 5$, $c = 2$, $m = 500$, $M = 5,000$.

APPENDIX B: Microbiological Requirements:

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TABLE 1: MICROBIOLOGICAL REQUIREMENTS FOR SEA FOODS

Sl. No	Name of the product	Total plate count	<i>E. Coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella & Shigella</i>	<i>Vibro Cholerae</i>	<i>Vibro Parahaemolyticus</i>	<i>Clostridium perfringens</i>
1	Frozen Shrimps of prawns							
	Raw	Not more than five lakh/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-
	Cooked	Not more than one lakh/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-
2	Frozen Lobsters							
	Raw	Not more than five lakh/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-
	Cooked	Not more than one lakh/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-
3	Frozen	Not more than five lakh/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-
4	Frozen finfish	Not more than five lakh/gm	Absent in 25 gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-
5	Frozen fish fillets or	Not more than five lakh/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-

Sl. No	Minced fish flesh or mixtures thereof Name of the product	Total plate count	<i>E. Coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella & Shigella</i>	<i>Vibro Cholerae</i>	<i>Vibro Parahaemolyticus</i>	<i>Clostridium perfringens</i>
1	Dried Shark fins	Not more than five lakh/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-
2	Salted fish/ dried salted fish	Not more than five lakh/gm	Not more than 20/gm	Not more than 100/gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-
3	Canned finfish	Nil	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm
4	Canned shrimp	Nil	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm			-
5	Canned sardines or sardine type products	Nil	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-
6	Canned salmon	Nil	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-
7	Canned crab meat	Nil	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	-

TABLE 3: MICROBIOLOGICAL PARAMETER FOR SPICES

Sl. No	Requirements	Caraway (Shiahjira)	Cardomom (Elaichi)	Chillies and Capsicum (Lalmirchi)	Cinnamon (dalchini)	Cassia (Taj)	Cassia (Taj)	Coriander (Dhania)
1	Total plate count	-	-	-	-	-	-	-
2	Coliform count	-	-	-	-	-	-	-
3	<i>E. Coli</i>	-	-	-	-	-	-	-
4	<i>Salmonella</i>	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm
5	<i>Shigella</i>	-	-	-	-	-	-	-
6	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-
7	Yeast and Mould Count	-	-	-	-	-	-	-
8	Anaerobic Spore Count	-	-	-	-	-	-	-
9	<i>Listeria monocytogenes</i>	-	-	-	-	-	-	-

Sl. No	Requirements	Cumin (Zeera, Kalunji)	Fennel (Saunf)	Fenugreek (Methi)	Ginger (Sonth, Adrak)	Mace (Jaipatri)	Mustard (Rai, Sarson)	Nutmeg (Jaiphal)
1	Total plate count	-	-	-	-	-	-	-
2	Coliform count	-	-	-	-	-	-	-
3	<i>E. Coli</i>	-	-	-	-	-	-	-
4	<i>Salmonella</i>	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm
5	<i>Shigella</i>	-	-	-	-	-	-	-
6	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-
7	Yeast and Mould Count	-	-	-	-	-	-	-
8	Anaerobic Spore Count	-	-	-	-	-	-	-
9	<i>Listeria monocytogenes</i>	-	-	-	-	-	-	-

Sl. No	Requirements	Papper (Kalimirsch)	Poppy (Khas Khas)	Saffron (Kesarf)	Turmeric (Haldi)	Curry powder	Mixed masala	Aniseed (Saunf)
1	Total plate count	-	-	-	-	-	-	-
2	Coliform count	-	-	-	-	-	-	-
3	<i>E. Coli</i>	-	-	-	-	-	-	-
4	<i>Salmonella</i>	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm
5	Shigella	-	-	-	-	-	-	-
6	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-
7	Yeast and Mould Count	-	-	-	-	-	-	-
8	Anaerobic Spore Count	-	-	-	-	-	-	-
9	<i>Listeria monocytogenes</i>	-	-	-	-	-	-	-

[illegible]

TABLE 2: MICROBIOLOGICAL PARAMETER FOR MILK PRODUCTS

Sl. No	Requirements	Ice cream/ Frozen Dessert/ Milk Lolly/ Ice Candy/ Dried Ice cream mix	Cheese/ processed cheese/ Cheese Spread/ All other Cheeses	Evaporated Milk Sweetened Condensed Milk	Sweetened Condensed Milk	Butter	Butter oil/ Butter Fat/ Ghee	Yoghurt/ Dahi
1	Total plate count	Not more than 2,50,000/ gm	Not more than 50,000/ gm	Not more than 500/ gm	Not more than 500/ gm	Not more than 5000/ gm	Not more than 5000/ gm	Not more than 10,00,000/ gm
2	Coliform count	Not more than 10/ gm	Absent in 0.1 gm	Absent in 0.1 gm	Absent in 0.1 gm	Not more than 5/ gm	Absent in 1.0 gm	Not more than 10/ gm
3	E. Coli	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1.0 gm
4	Salmonella	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm
5	Shigella	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm	Absent in 25 gm
6	Staphylococcus aureus	Absent in 1 gm	Absent in 1 gm	Not more than 100/ gm	Not more than 100/ gm	Absent in 1 gm	Absent in 1 gm	Not more than 100/ gm
7	Yeast and Mould Count	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Not more than 10/ gm	Not more than 20/ gm	Absent in 1 gm	Not more than 100/ gm
8	Anaerobic Spore Count	Absent in 1 gm	Absent in 1 gm	Not more than 5/gm	Absent in 1 gm	Not more than 5/gm	Absent in 1 gm	Absent in 1 gm
9	Listeria monocytogens	Absent in 1 gm	Cheese other than hard cheese: Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm	Absent in 1 gm

TABLE 4: Microbiological requirements of food products given below:-

Sl.No	Products	Parameters	Limits
1	Thermally processed fruits and vegetable products	Total plate count	Not more than 50/ ml
		Incubation at 37°C for 10 days and 55°C for 7 days	No changes in p ^H
2	a) Dehydrated fruits and vegetables b) Soup powders c) Desiccated coconut powder d) Table olives e) Raisins f) Pistachio nuts g) Dates h) Dry fruits and nuts	Total plate count	Not more than 40,000/ gm
3	Carbonated beverages, ready – to – serve beverages including fruit beverages	(a) Total plate count	Not more than 50 cfu/ ml
		(b) Yeast and mould count	Not more than 2.0 cfu/ ml
		(c) Coli form count	Absent in 100ml
4	Tomato products		
	Tomato juices and soups	(a) Mould Count	Positive in not more than 40.0 % of the field examined
	Tomato puree and paste	(b) Yeast spores	Not more than 125 per 1/60 c.m.m
	Tomato ketchup and tomato Sauce	(a) Mould Count	Positive in not more than 60.0 % of the field examined
		(b) Mould count	Positive in not more than 40.0 % of the field examined
		(c) Yeast & spores	Not more than 125 per 1/60 c.m.m
		(d) Total plate count	Not more than 10000 / ml

5	Jam / Marmalade / Fruit jelly / Fruit Chutney and Saucesd	Total plate count	Positive in not more than 40.0 % of the field examined
		Yeast and spores	Not more than 125 per 1 / 60 c.m.m
6	Other fruits and vegetables products covered under item A. 16 of Appendix B	Yeast and mold count	Positive in not more than 100 count / gm
7	Frozen fruits and vegetables products	Total plate count	Not more than 40,000/gm
8	Preserves	Mould count	Absent in 25 gm / ml
9	Pickles	Mould count	Absent in 25 gm / ml
10	Fruits cereal Flakes	Mould count	Absent in 25 gm / ml
11	Candied and crystallized or Glazed Fruit and peel	Mould count	Absent in 25 gm / ml
12.	(a) All Fruit and Vegetable products and ready – to – serve Beverages including Fruit Beverages and synthetic products covered under A. 16 of Appendix B.	a. Flat Sour Organisms	i) Not more than 10000 cfu/ rm for those products which have P ^H less than 5.2 ii) Nil for those products which have P ^H more than 5.2
	b) Table olives	b. <i>Staphylococcus aureus</i>	Absent in 25 gm/ ml
	c) Raisins	c. <i>Salmonella</i>	Absent in 25 gm/ ml
	d) Pistachio nuts	d. <i>Shigella</i>	Absent in 25 gm/ ml
	e) Dates	e. <i>Clostridium botulinum</i>	Absent in 25 gm/ ml
	f) Dry fruits and nuts	f. E. Coli	Absent in 1 gm/ ml
	g) Vinegars	g. <i>Vibrio Cholera</i>	Absent in 25 gm/ ml

LECTURE-29

Food laws & Standards- FAO, Codex Alimentarius, ISO, Indian food laws and standards, Prevention of Food adulteration (PFA)act, Fruit Products Order(FPO), Meat product order (MPO), Cold storage order (CSO),BIS, Agmark.

STANDARD:

- ⊙ "Standard" ("*norme*"): broad meaning in the general language
- ⊙ More restrictive meaning in official and technical languages: refers to something voluntary
- ⊙ Mandatory standards are called "technical regulations" ("*règlements techniques*")
- ⊙ Standards provide "rules guidelines, or characteristics for activities or their results, aimed at the achievement of the optimum degree of order in a given context" .
- ⊙ Standards include various layers of "quality insurance institutions": rule making, conformity assessment, accreditation.

STANDARDIZATION SYSTEMS IN INDIA:

- ⊙ In the food and agriculture sector there are number of organizations responsible for the formulation of Standards and monitoring their quality. These can be generally classified in two systems as under.
- 1. Compulsory- all orders under Essential Commodities Act 1955.
- 2. Voluntary based product certification viz.ISI mark of BIS & Agmark.

Compulsory legislations:

- ⊙ Prevention of Food Adulteration Act 1954.
- ⊙ Fruit product Order, 1955.
- ⊙ Vegetable oil products Order.
- ⊙ Solvent extraction oil Order.
- ⊙ Meat product control Order, 1973.
- ⊙ Edible oil packaging.
- ⊙ Milk and milk product Order.
- ⊙ Exports Act.
- ⊙ Standard of weight & measure Act.

Voluntary based product Certification:

- Bureau of Indian Standard Act 1986.
- Agmark Grading & Marketing & Rules, 1937(Agmark).
- Eco-mark.
- ISO Standards.

Inspection:

- ⊙ An inspection is, most generally, an organized examination or formal evaluation exercise.
- ⊙ It involves the measurements, tests, and gauges applied to certain characteristics in regard to an object or activity.

- ⦿ The results are usually compared to specified requirements and standards for determining whether the item or activity is in line with these targets. Inspections are usually non-destructive.
- ⦿ A surprise inspection tends to have different results than an announced inspection. Leaders seeking to discover how well lower echelons in their organization are typically doing sometimes drop in unannounced to see what is going on and what conditions are.
- ⦿ When an inspection is scheduled in advance, it gives people a chance to cover up or fix mistakes.
- ⦿ A surprise inspection, therefore, gives inspectors a better picture of the typical state of the inspected object than an announced inspection.

Traceability :

- ⦿ Traceability refers to the completeness of the information about every step in a process chain.
- ⦿ Traceability is the ability to verify the history, location, or application of an item by In food processing (meat processing, fresh produce processing), the term traceability refers to the recording through means of barcodes or tags & other tracking media, all movement of product and steps within the production process.
- ⦿ One of the key reasons this is such a critical point is in instances where an issue of contamination arises, and a recall is required.
- ⦿ Where traceability has been closely adhered to, it is possible to identify, by precise date/time & exact location which goods must be recalled, and which are safe, potentially saving millions of dollars in the recall process.
- ⦿ Traceability within the food processing industry is also utilised to identify key high production & quality areas of a business, versus those of low return, and where points in the production process may be improved means of documented recorded identification.

Authentication:

- ⦿ Authentication is the act of Product authentication.
- ⦿ A Security hologram label on an electronics box for authentication Counterfeit products is often offered to consumers as being authentic.
- ⦿ Counterfeit consumer goods such as electronics, music, apparel, and Counterfeit medications have been sold as being legitimate. Efforts to control the supply chain and educate consumers to evaluate the packaging and labeling help ensure that authentic products are sold and used.

Certification:

- ⦿ Certification refers to the confirmation of certain characteristics of an object, person, or organization. This confirmation is often, but not always, provided by some form of external review, education, or assessment..
- ⦿ The other most common type of certification in modern society is product certification. This refers to processes intended to determine if a product meets minimum standards, similar to quality assurance.

- ☉ Product certification or product qualification is the process of certifying that a certain product has passed performance and quality assurance tests or qualification requirements stipulated in regulations such as a building code and nationally accredited test standards, or that it complies with a set of regulations governing quality and minimum performance requirements.

THE PREVENTION of FOOD ADULTERATION Act 1954:

- ☉ The most important compulsory legislation in our country in the area of food products in the prevention of Food Adulteration Act. (PFA-1954).
- ☉ Act is the basic statutory regulation is intended to protect the common consumer against the supply of adulterated food products.
- ☉ The Act makes provision for preservation of adulteration of food products and lays down that no person shall manufacture for sale, store, distribute any adulterated or misbranded food products not conforming to the standards laid down under the Rules.
- ☉ These standards are of minimum quality and are intended to ensure safety in the consumption of these food products and safe guarding against harmful impurities contaminations and adulteration etc.
- ☉ Provision of this act are mandatory and contravention to these rules leads to both fine and imprisonment.
- ☉ Central Committee for food standards (C.C.F.S.) and its various Subcommittees under the Directorate General of Health Services (D.G.H.S) Ministry of Health and Family Welfare is responsible for operation and enforcement of the Act.
- ☉ Various interests concerned with Food Standards including consumer interests have representations in this Committee.

OBJECTIVES OF PFA:

1. To protect the public from poisonous and harmful foods.
 2. To prevent the sale of substandard foods.
 3. To protect the interests of the consumers by eliminating fraudulent practices.
- According to the Act, an article of the food shall be deemed to be adulterated.
- ☉ If the article sold by a vendor is not of the nature, substance or quality demanded by the purchaser and as it is represented to be.
 - ☉ If any inferior or cheaper substance has been substituted wholly or in part for the article.
 - ☉ If the article had been prepared, packed or kept under unsanitary conditions whereby it has become contaminated or injurious to health.
 - ☉ If the article contains any poisonous or other ingredient which renders its contents injurious to health.
 - ☉ If the container of the article is composed of any poisonous or deleterious substance which renders it contents injurious to health.
 - ☉ If any coloring matter other than as prescribed and in amounts not within the prescribed limits of variability is present in the article.
 - ☉ If the article contains any prohibited preservative or permitted in excess of the prescribed limits.

- ⊙ If the quality or purity of the article falls below the prescribed standard or its constituents are present in quantities, which are in excess of the prescribed limits variability.

FRUIT PRODUCT ORDER:

- ⊙ Fruits and Vegetable Processing Sector is regulated by the Fruit Products Order, 1955 (FPO), which is administrated by the ministry of Food processing Industries, Government of India.
- ⊙ The license shall be in force for such period as may be specified therein, usually for three/five or ten years.
- ⊙ For the renewal of a license application shall be submitted at least one month before the expiry of the period of the license.
- ⊙ The licensing officer may by order refuse to grant a license to any applicant giving a brief statement of doing so.
- ⊙ The manufacturer may appeal to the Central Govt against such refusal within a period of thirty days of the receipt of the order and the decision of the Central Government shall be final.
- ⊙ Every manufacturer shall manufacture fruit products in conformity with the sanitary requirements and the appropriate standard of the quality and composition specified in the Second schedule of this Order.
- ⊙ The Order lays down specifications and quality control requirements on the production and marketing of the fruits and vegetables food products.
- ⊙ With regard to packing, marking and labeling of the containers of fruits products, the following requirements have to be fulfilled.
 1. Every container in which any fruit product is packed shall bear the label as approved by the Licensing Officer.
 2. The bottle shall be so sealed that it cannot be opened without destroying the license number of the special identification mark of the manufacturer to be displayed on the top or neck of the bottle or in case of tin, barrel or other container the license number shall be exhibited prominently on the side label of such tin or be embossed thereon.
- ⊙ The label should not contain any statement, claim, design or device, which is false or misleading in any particular concerning the fruit products ect.
- ⊙ Every beverage should contain at least 25% of fruit juice in its composition otherwise it will be treated as synthetic and it shall immediately precede such name of the product and in case of artificial flavors the 'Artificially Flavored' shall also be added.
The order lays down specifications and quality control requirements on the production and marketing of the following food products:
- ⊙ Fruit juice, Pulp concentrates, Squashes, Cordials, Crush, Fruit Syrup, Nectar, Aerated water containing-Fruit Juice or Pulp and Ready to serve Beverages.
- ⊙ Fruits Nectar, Canned Mango Pulp (Natural and Sweetened), and Sweetened Aerated Waters with no Fruit Pulp or containing less than 10% of fruit juice or Pulp.
- ⊙ Sweetened Aerated Water with 10 or more of fruit juice of fruit pulp.
- ⊙ Barley Waters (lemon, orange, grape fruits etc.

- ⊙ Synthetic Syrup, Ginger Cocktail, Ginger Beer, Ginger Ale and Sharbats.
- ⊙ Bottled and canned fruits and vegetables.

MEAT FOOD PRODUCTS ORDER:

- ⊙ Meat Food Products Order, 1973 (MFPO) promulgated under the provisions of Essential Commodities Act, 1955 provides for sanitary and other requirements, limits of heavy metals, preservatives, insecticides, residue, ect., for meat food products.
- ⊙ Under the provision of MFPO all manufacturers of meat food products engaged in the business of manufacturing, packing, repacking, relabeling meat food products meant for sale are licensed but excluding those manufactures such products for consumption on the spot like a restaurant, hostel, boarding house, snack bar, eating house or any other similar establishment.

The main objectives of the MFPO, 1973 are

- ⊙ To regulate production and sale of meat products through licensing of manufacturer.
- ⊙ Enforce sanitary and hygienic conditions prescribed for production of wholesome meat food products.
- ⊙ Exercise strict quality control at all stages of production of meat food products, fish products including chilled poultry ect.
- This Order was being implemented by Department of Rural Development in the Ministry of Rural Area and Employment.
- As per the recent amendment to the Allocation of Business, Ministry of Agriculture (Deptt. Of Agriculture and Cooperation) would now be the Administrative Ministry for this Order.

MILK AND MILK PRODUCTS ORDER:

- ⊙ Milk and Milk Products Order, 1992 administrated by the Department of Animal Husbandry and Dairying under Ministry of Agriculture was promulgated on 9th June, 1992 under the provision of Section 3 of the Essential Commodities Act, 1955 with a view to maintain an increased supply of liquid milk of desired quality to the general public.
- ⊙ This order regulated production, supply and distribution of milk and milk products throughout the country.
- ⊙ This order also seeks to ensure the observance of sanitary requirements for dairies, machinery and premises, and quality control standards for milk and milk products.
- ⊙ There is no restriction on setting up of new dairy units and expansion in the milk processing capacity, while noting the requirement of registration is for enforcing the prescribed Sanitary and Hygienic Conditions, Quality and Food Safety Measures as specified in 5th schedule of MMPO-1992.
- ⊙ In order to comply the provision of Para 5 (5) (B) of MMPO-92, two inspection agencies i.e. National Productivity Council (NPC) and Export Inspection Council (EIC) of India have been notified for annual inspection of registered dairy units, on rotation basis.

BUREAU OF INDIAN STANDARD:

- ⊙ During the pre independence period, standardization activity was sporadic and confined mainly to a few Government purchasing organization.
- ⊙ However immediately after independence, economic development through coordinated utilization of resources was called for and the Government recognized the role for standardization in gearing industry to competitive efficiency and quality production.
- ⊙ The Indian Standards Institution (ISI) was, therefore, set up in 1947 as a registered society, under a Government of Indian resolution.
- ⊙ The Indian Standards Institution gave the nation the standards it needed for nationalization, orderly industrial and commercial growth, quality production and competitive efficiency.
- ⊙ In 1986 the Government recognized the need for strengthening this National standards body due to fast changing socio-economic scenario and according it a statutory status.
- ⊙ Thus came the bureau of Indian Standards Act 1986 and on 1st April 1987, newly formed BIS took over staff assets, liabilities and functions of erstwhile ISI.
- ⊙ Through this change over, the Government envisage building of the climate of quality culture and consciousness and greater participation of consumers in formulation and implementation of National quality control.

OBJECTIVES:

- ⊙ Harmonious development of standardization, marketing and quality certification.
- ⊙ To provide new thrust to standardization and quality control.
- ⊙ To evolve a national strategy for according recognition to standard and integrating them with growth and development of production and exports.

A.G.M.A.R.K(GRADING AND MARKETING ACT):**OBJECTIVES:**

Promotion of grading and Standardization of agriculture and allied commodities under Agriculture Produce (Grading & Marking) Act, 1937.

SALIENT FEATURES:

Quality standards for agricultural commodities are framed based on their intrinsic quality.

Food safety factors are being incorporated in the standards to complete in world trade.

Standards are being harmonized with international standards keeping in view the WTO requirements.

Certification of agricultural commodities is carried out for the benefit of produce/manufacturer and consumer.

Certification of adulteration prone commodities viz. Butter, Ghee, Vegetable Oils, Ground-spices, Honey, Wheat Atta etc. is very popular.

Blended Edible vegetable Oils and Fat Spread are compulsorily required to be certified under agmark.

Definition: RECALL:

Firm's removal of marketed product that the Food & Drug Administration considers to be in violation of the law it administers and against which the agency would initiate legal action; e.g., seizure.

Does not include a market withdrawal or a stock recovery.

Definition: MARKET WITHDRAWAL:

Firm's removal or correction of a distributed product which involves a minor violation that would not be subject to legal action by FDA or which involves no violation, e.g., normal stock rotation practices, routine equipment adjustments and repairs, etc.

Definition: STOCK RECOVERY:

Firm's removal or correction of a product that has not been marketed or that has not left the direct control of the firm, e.g., the product is located on premises owned by, or under the control of, the firm and no portion of the lot has been released for sale or use.

Definition: CORRECTION:

Repair, modification, adjustment, re-labeling, destruction, or inspection of a product and/or the promotional literature which causes the product to be violative, without its physical removal to some other location.

RECALLS:

May be undertaken voluntarily and at any time.

In response to a formal request by FDA.

Means of protecting the public health and well-being.

Retrieving products that present a risk of injury or gross deception or are otherwise defective.

When a firm decides to initiate a product removal or correction, it should proceed with the action and not wait for FDA to determine whether that action is a recall, conduct a hazard evaluation, classify the recall, and review the strategy for that recall.

The recall of an FDA-regulated product is the responsibility of both FDA and the firm responsible for the manufacture of that product. This is essential to define not only FDA's role in product recalls, but industry's as well because ultimately the recall procedures that a firm follows actually determines the success or failure of a particular recall.

A voluntary recall does not preclude FDA from invoking any of its regulatory powers.

Because a recall similar to seizure in basis and effect, injunction, criminal prosecution, and/or other sanctions may be also appropriate in a recall situations.

A firm can first make an initial decision whether an action is a recall; however, FDA makes the final decision as to whether the firm's removal or correction constitutes an FDA recall.

FDA has the responsibility for deciding when a firm's action is or is not a recall.

TYPES OF RECALLS:

FIRM INITIATED (most common):

Initiated by a firm independently and under any circumstances to remove or correct a distributed product.

Initiated by a firm when informed by the FDA that the product in question violates the law, but the agency has not specifically requested a recall.

FDA REQUESTED –

The Commissioner of FDA or his designee may request a firm to initiate a recall when the following determinations have been made:

Urgent situation;

Risk of illness, injury, or gross consumer deception;

Firm has not initiated a recall; and/or

Necessary to protect public health and welfare.

***Definition:* RECALLING FIRM**

The firm who initiates a recall, or in the case of an FDA-requested recall, the firm that has primary responsibility for the manufacturing and marketing of the product to be recalled.

***Definition:* RECALL STRATEGY:**

A planned specific course of action to be taken in conducting a specific recall, which addresses the depth of recall, need for public warning, and extent of effectiveness checks for the recall.

The planned course of action to be carried out by the firm in the achievement of its recall goals. The FDA will review and/or recommend changes to the firm's recall strategy, as appropriate.

RECALL STRATEGY FACTORS:

Results of firm's health hazard evaluation.

Ease in identifying the product.

Degree to which the product's deficiency is obvious to the consumer or user.

Degree to which the product remains unused in the marketplace.

Continued availability of essential products.

HEALTH HAZARD EVALUATION:

Evaluation by FDA scientists of the threat to health presented by a product, including its labeling and/or promotional literature, that is being recalled or considered for recall.

HEALTH HAZARD FACTORS:

Any disease or injury has occurred.

Any contributing factors.

Assessment of hazard to various segments of the population.

Assessment of the degree of seriousness.

Assessment of the likelihood of occurrence.

Assessment of the consequences of occurrence.

DEPTH OF RECALL:

Level in the distribution chain to which the recall is to be extended.

Depends on the product's degree of hazard and the extent of distribution.

Consumer or user level.

Retail level.

Wholesale level.

***Definition:* CONSIGNEE:**

Anyone who received, purchased, or used the product being recalled.

RECALL COMMUNICATIONS:

Should be brief and to the point.

Clearly identify the product.

Concisely explain the reason for the recall and the hazard involved.

Provide specific instruction on what should be done with respect to the recalled product(s).

Convey the name of the recalled product.

Further distribution or use of any remaining product should cease immediately.

When appropriate, that the direct account should conduct a sub-recall.

Instructions regarding what to do with the product.

Should not be diluted or camouflaged by irrelevant qualifications, promotional materials, or any other statement or information that may detract from the message. Provide a means for the recipient to report back to the recalling firm.

RES: Recall Enterprise System:

Automated system

Track Recalls Nationwide

Consistency in recall reporting

Time efficient

Real time agency awareness

Real time public awareness (Internet).

RECALL CLASSIFICATIONS:

CLASS I: A situation in which there is a reasonable probability that the use of, or exposure to, a violative product will cause serious adverse health consequences or death.

PUBLIC WARNINGS:

Purpose is to alert the public health that the recalled product presents a serious hazard to health.

Reserved for urgent situations where other means for preventing use appear inadequate.

Issued through the general news media, and/or the specialized news media, or to specific segments of the population.

CLASS II: A situation in which use of, or exposure to, a violative product may cause temporary or medically reversible adverse consequences or where the probability of serious adverse health consequences is remote.

CLASS III: A situation in which use of, or exposure to, a violative product is not likely to cause adverse health consequences.

PUBLIC NOTIFICATION OF RECALL:

Weekly FDA Enforcement Report:

Contains a descriptive listing of each new recall, its classification, and the specific action being taken by the recalling firm.

Does not contain a firm's product removals or corrections, which are classified as market withdrawals or stock recoveries.

EFFECTIVENESS CHECKS:

Conducted by the recalling firm.

Verify that all consignees have received notification about the recall and have taken appropriate action.

If not done, the firm is not meeting its obligation and responsibility to the consumer.

RECALL STATUS REPORTS:

Recalling firm is requested to submit periodic recall status reports so that an assessment can be made of the progress of the recall.

Frequently will be determined by the relative urgency of the recall.

Should contain the following information:

Number of consignees notified, date, and method.

Number of responders and quantity on hand.

Number of non-responders.

Amount product returned or corrected and quantity accounted for.

Number and results of effectiveness checks.

Estimated time frames for completion.

LEVEL OF AUDIT CHECKS:

Percentage of total number of consignees contacted:

Level A – 100%

Level B – greater than 10% but less than 100%

Level C – 10%

Level D – 2%

Level E – No effectiveness checks.

FDA RECALL AUDIT CHECK PROGRAM:

Audit checks determine the adequacy of the firm's effectiveness checks.

Audit checks are decided upon after evaluating the recalling firm's strategy.

Audit checks are conducted by:

- Personal visits
- Telephone calls

TERMINATION OF RECALL:

Occurs when FDA has determined that all reasonable efforts have been made to remove or correct the violative product in accordance with the recall strategy and proper disposition has been made according to the degree of hazard.

LECTURE-30

Non permitted food additives-Allura red AC, Aspartame, amaranth, Benzoic acid, brilliant black, Butylated Hydroxy-anisole, Calcium benzoate, Calcium sulphite.

Allura Red AC

Allura Red AC is a red azo dye. It has several names including Allura Red, Food Red 17, C.I. 16035, FD&C Red 40, 2-naphthalenesulfonic acid. It is used as a food dye and has the E number E129. Allura Red AC was originally introduced in the United States as a replacement for the use of amaranth as a food coloring.

In Europe, Allura Red AC is not recommended for consumption by children. It is banned in Denmark, Belgium, France and Switzerland, and was also banned in Sweden.

In the United States, Allura Red AC is approved by the Food and Drug Administration (FDA) for use in cosmetics, drugs, and food. It is used in some tattoo inks and is used in many products, such as soft drinks, children's medications, and cotton candy.

Food coloring in snacks, sauces, preserves, soups, wine, cider, etc.

Avoid if suffering from asthma, rhinitis (including hay fever), or urticaria (hives).

Aspartame

Aspartame is an artificial, non-saccharide sweetener used as a sugar substitute in some foods and beverages. In the European Union, it is codified as E951. Aspartame is a methyl ester of the aspartic acid/phenylalanine dipeptide. It was first sold under the brand name NutraSweet; since 2009 it also has been sold under the brand name AminoSweet.

Because its breakdown products include phenylalanine, aspartame must be avoided by people with the genetic condition phenylketonuria (PKU).

Aspartame, an artificial sweetener, is approximately 200 times sweeter than sucrose, or table sugar.

Upon ingestion, aspartame breaks down into natural residual components, including aspartic acid, phenylalanine, methanol, and further breakdown products including formaldehyde and formic acid, accumulation of the latter being suspected as the major cause of injury in methanol poisoning.

Human studies show that formic acid is excreted faster than it is formed after ingestion of aspartate. In some fruit juices, higher concentrations of methanol can be found than the amount produced from aspartame in beverages.

Amaranth:

Amaranth, (E123, C.I. Food Red 9, Acid Red 27, Azorubin S, or C.I. 16185), is a dark red to purple azo dye once used as a food dye and to color cosmetics. Since 1976 it has been banned in the United States by the Food and Drug Administration (FDA) as it is a suspected carcinogen.

It usually comes as a trisodium salt. It has the appearance of reddish-brown, dark red to purple water-soluble powder that decomposes at 120°C without melting.

As a food additive it has E number E123. Like all azo dyes.

Used as food coloring in wine, spirits, fish roe.

Banned in the U.S. Avoid if suffer from asthma, rhinitis, urticaria or other allergies

Benzoic acid:

Benzoic acid is a colorless crystalline solid and the simplest aromatic carboxylic acid. The name derived from gum benzoin, which was for a long time the only source for benzoic acid. Its salts are used as a food preservative and benzoic acid is an important precursor for the synthesis of many other organic substances. The salts and esters of benzoic acid are known as benzoates

Biology and health effects

Benzoic acid is present as part of hippuric acid in urine of mammals, especially herbivores. Humans produce about 0.44 g/L hippuric acid per day in their urine, and if the person is exposed to toluene or benzoic acid it can rise above that level.

For humans, the World Health Organization's International Programme on Chemical Safety (IPCS) suggests a provisional tolerable intake would be 5 mg/kg body weight per day.

Too much benzoic acid, which is known to affect the liver and kidney, along with more serious issues like excessive cyclamate. Benzoic acid activates bromelain.

Brilliant Black:

Brilliant Black BN (Brilliant Black PN, Brilliant Black A, Black PN, Food Black 1, Naphthol Black, C.I. Food Brown 1, or C.I. 28440) is a synthetic black diazo dye. It is soluble in water. It usually comes as tetrasodium salt. It has the appearance of solid, fine powder or granules. Calcium and potassium salts are allowed as well.

When used as a food dye, its E number is E151. It is used in food decorations and coatings, desserts, sweets, ice cream, mustard, red fruit jams, soft drinks, flavored milk drinks, fish paste, lumpfish caviar and other foods.

It appears to cause allergic or intolerance reactions, particularly amongst those with an aspirin intolerance. It is a histamine liberator, and may worsen the symptoms of asthma.

E151 has been banned in many countries.

Potentially dangerous to asthmatics, probable cause for ADD in children and may cause urticaria and problem to rhinitis sufferers. Also known to interfere with some digestive enzymes.

Butylated hydroxyanisole (BHA)

It is prepared from 4-methoxyphenol and isobutylene. It is a waxy solid used as a food additive with the E number E320. The primary use for BHA is an antioxidant and preservative in food, food packaging, animal feed, cosmetics, rubber, and petroleum products. BHA also is commonly used in medicines, such as isotretinoin, lovastatin, and simvastatin.

Preservative, particularly in fat-containing foods, confectionery, meats.

The International Agency for Research on Cancer says BHA is possibly carcinogenic to humans. BHA also interacts with nitrites to form chemicals known to cause changes in the DNA of cells.

LECTURE-31

Non permitted food additives- Monosodium glutamate (MSG) Ponceau 4R, Conchineal Red A, Potassium benozoate, Potassium nitrate, Propyl p-hydroxybenzoate, propylparaben, and paraben.

Monosodium glutamate:

Monosodium glutamate, also known as sodium glutamate or MSG, is the sodium salt of glutamic acid, one of the most abundant naturally occurring non-essential amino acids. It has been classified by the U.S. Food and Drug Administration as generally recognized as safe (GRAS) and by the European Union as a food additive.

MSG has the HS code 29224220 and the E number E621.

Trade names of monosodium glutamate include AJI-NO-MOTO®, *Vetsin*, and *Ac'cent*.

Usage: As a flavor enhancer and in the right amount, MSG has the ability to enhance other taste-active compounds balancing and rounding the overall taste of certain dishes. MSG mixes well with meat, fish, poultry, many vegetables, sauces, soups and marinades, and increases the overall preference of certain foods like beef consomme.

The "MSG symptom complex" was originally termed as the "Chinese Restaurant Syndrome".

In normal conditions, humans have the ability to metabolize glutamate that has a very low acute toxicity. Has been known to cause pressure on the head, seizures, chest pains, headache, nausea, burning sensations, and tightness of face. Many baby food producers have stopped adding MSG to their products.

Ponceau 4R

Also known as C.I. 16255, Conchineal Red A, C.I. Acid Red 18, Brilliant Scarlet 3R, Brilliant Scarlet 4R, New Coccine, SX purple is a synthetic colourant that may be used as a food colouring. It is denoted by E Number E124.

Ponceau 4R is a red azo dye which can be used in a variety of food products, and is usually synthesized from aromatic hydrocarbons from petroleum.

Health effects: People who suffer from asthma, rhinitis or urticaria may find their symptoms become worse following consumption of foods containing this coloring.

Because it is an azo dye, it may elicit intolerance in people allergic to salicylates (aspirin). Additionally, it is a histamine liberator, and may intensify symptoms of asthma.

Acceptable Daily Intake (ADI) for Ponceau 4R from 4 mg/kg to 0.7 mg/kg bodyweight per day.

Potassium Benzoate

Potassium benzoate (E212), the potassium salt of benzoic acid, is a food preservative that inhibits the growth of mold, yeast and some bacteria. It works best in low-pH products, below 4.5, where it exists as benzoic acid.

Acidic foods and beverages such as fruit juice (citric acid), sparkling drinks (carbonic acid), soft drinks (phosphoric acid), and pickles (vinegar) may be preserved with potassium benzoate. It is not recommended for consumption by children.

Potassium benzoate is also used as the whistle in many fireworks.

In combination with ascorbic acid (vitamin C), sodium and potassium benzoate may form benzene, a known carcinogen.

Preservative in many foods, including drinks, low-sugar products, cereals, meat products.

Can temporarily inhibit function of digestive enzymes and may deplete levels of the amino acid glycine. Should be avoided by those with hay fever, hives, and asthma.

Propylparaben

Propyl paraben is propyl ester of *p*-hydroxybenzoic acid, occurs as a natural substance found in many plants and some insects, although it is manufactured synthetically for use in cosmetics, pharmaceuticals and foods. It is a preservative typically found in many water-based cosmetics, such as creams, lotions, shampoos and bath products. As a food additive, it has the E number E216.

Sodium propyl *p*-hydroxybenzoate, is used similarly as a food additive and as an anti-fungal preservation agent. Its E number is E217.

Parabens cause skin irritation and contact dermatitis and rosacea in individuals with paraben allergies, a small percentage of the general population. Causes breast cancer

LECTURE-32

Non permitted food additives- Saccharin & its Na, K and Ca salts, Sodium metabisulphite, Sodium sulphite, Stannous chloride (tin), Sulphur dioxide, Sunset Yellow FCF, Orange Yellow S, tartrazine.

Sachharin & its Na, K and Ca salts:

Saccharin is an artificial sweetener. The basic substance, benzoic sulfilimine, has effectively no food energy and is much sweeter than sucrose, but has a bitter or metallic aftertaste, especially at high concentrations. It is used to sweeten products such as drinks, candies, cookies, medicines, and toothpaste.

Sweetener in diet, and no-sugar products. The International Agency for Research on Cancer has concluded that saccharin is possibly carcinogenic to humans.

Sodium metabisulphite:

Preservative and antioxidant. May provoke life-threatening asthma.

Sodium sulphite:

Preservative used in wine-making and other processed foods.

Sulphites have been associated with triggering asthma attacks. Most asthmatics are sensitive to sulphites in food.

Stannous chloride (tin)

Antioxidant and color-retention agent in canned and bottled foods, fruit juices.

Acute poisoning has been reported from ingestion of fruit juices containing concentrations of tin greater than 250 mg per liter.

Sulphur dioxide

Preservative. Sulphur dioxide reacts with a wide range of substances found in food, including various essential vitamins, minerals, enzymes and essential fatty acids. Adverse reactions: bronchial problems particularly in those prone to asthma, hypotension (low blood pressure), flushing tingling sensations or anaphylactic shock. International Labour Organization says to avoid E220 if you suffer from conjunctivitis, bronchitis, emphysema, bronchial asthma, or cardiovascular disease.

Sunset Yellow FCF, Orange Yellow S:

Sunset Yellow FCF (also known as Orange Yellow S, FD&C Yellow 6 or C.I. 15985) is a synthetic yellow azo dye, manufactured from aromatic hydrocarbons from petroleum. It is denoted by E Number E110. It induce allergic reactions.

Food coloring. Some animal studies have indicated growth retardation and severe weight loss. People with asthma, rhinitis, or urticaria should avoid this product

Tartrazine:

Yellow food colouring

May cause allergic reactions and asthmatic attacks and has been implicated in bouts of hyperactivity disorder in children. Those who suffer from asthma, rhinitis and urticaria may find symptoms worsen after consumption